CHARACTERISING THE MICROBIAL PROFILES OF VARIOUS RIVER SOURCES AND INVESTIGATING THE EFFICACY OF UV RADIATION TO REDUCE MICROBIAL LOADS FOR IMPROVED CROP SAFETY

By

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

The rivers used for the irrigation of fresh produce in the Western Cape have been under frequent investigation in recent years. Results have frequently shown that in rivers used for irrigation, the faecal coliform concentrations (*Escherichia coli)* frequently exceed the guideline limit of 1 000 colony forming units per 100 mL. These findings present a health risk for consumers of fresh produce. Ultraviolet (UV) radiation treatment has proven to offer some advantages for water disinfection over conventional treatment methods such as filtration and chemical treatments. However, this is not yet a common practice in South Africa. Knowledge gaps exist with regard to the efficacy of UV radiation on environmental strains of pathogenic microorganisms such as *Salmonella* species and *Listeria monocytogenes.* The aim of this study was to investigate the effect of low-pressure (LP) UV radiation on water obtained from various river water sources, in order to disinfect water used for irrigation purposes to ultimately reduce the risk of causing foodborne disease outbreaks from the consumption of contaminated fresh produce.

Four rivers in the Western Cape were sampled five times each between the wet winter and dry summer seasons, to establish the microbial and physico-chemical profiles of the rivers. These results were compared to the guideline limits. The samples were exposed to three doses (20, 40 and 60 $mJ.cm^{-2}$) of LP UV radiation at laboratory-scale. It was established that LP UV radiation was effective at reducing the microbial loads to non-detectable levels. Pathogenic microorganisms were successfully inactivated after a dose of 20 $mJ.cm^{-2}$. Heterotrophic Plate Count colony numbers were lowered more steadily, and therefore, showed greater resistance to treatment. Thirteen strains were isolated and stored for future experiments. It was suggested that a pre-treatment step be implemented to improve the physical quality of the river water prior to treatment.

Isolated strains of *E. coli* (n = 3), *Salmonella* species (n = 2) and *L. monocytogenes* (n = 8) were stored for further testing. The *L. monocytogenes* isolates (n = 8) were subjected to lineage typing experiments, where it was established that all isolates were lineage I. This lineage is most frequently associated with listeriosis. Extended-spectrum beta-lactamase (ESBL) testing indicated that none of the *Enterobacteriaceae* isolates (n=5) were ESBL-producers. All *Enterobacteriaceae* isolates showed resistance to tetracycline, ampicillin and trimethoprim-sulfamethoxazole. Resistance of *L. monocytogenes* isolates (n=5) was observed against trimethoprim-sulfamethoxazole, while four *L. monocytogenes* isolates showed resistance to ampicillin, penicillin and erythromycin. Multidrug resistance was reported for 90% of river water isolates (n=9).

Four different bag filter pore sizes (5, 20, 50 and 100 µm) were investigated to determine the most effective pre-treatment step to improve the UV transmission (UVT %) of the water. This experiment was performed on the 'worst case scenario' river, the Mosselbank River. Improvements in the total suspended solids, chemical oxygen demand and turbidity were reported, however, the extremely high total dissolved solids content (728.67 $mg.L^{-1}$) prevented a larger improvement in the UVT %. It was established that the 5 µm bag filter was the most effective pore size.

In the current study, LP UV radiation was successfully able to produce water of an acceptable standard for the irrigation of fresh produce. The physical quality of the water did not prevent a successful disinfection, but rather increased the exposure time required to deliver a specific dose and therefore, decreased efficiency. It was established that LP UV radiation is able to reduce pathogenic microorganisms to non-detectable levels. This method of disinfection, therefore, shows promise for full-scale application of irrigation water treatment.

UITTREKSEL

Die riviere wat vir die besproeiing van vars produkte in die Wes-Kaap gebruik word, is die afgelope paar jaar gereeld ondersoek. Resultate het getoon dat in sommige riviere die fekale koliforme konsentrasies (*Escherichia coli*) gereeld die riglynlimiet van 1 000 kolonievormende eenhede per 100 ml oorskry. Hierdie bevindings dui op 'n gesondheidsrisiko vir die verbruikers van vars produkte. Ultraviolet (UV) bestralingsbehandeling het bewys dat dit voordele bied vir die ontsmetting van water bo konvensionele behandelingsmetodes soos filtrasie en chemiese behandelings. Dit is egter nog nie 'n algemene gebruik in Suid-Afrika nie. Daar is kennisgapings met betrekking tot die doeltreffendheid van UV-bestraling op patogene mikroörganismes soos *Salmonella*-spesies en *Listeria monocytogenes.* Die doel van hierdie studie was om die effek van lae druk (LP) UV-bestraling op water, verkry uit verskillende rivierwaterbronne, te ondersoek, met die doel om water wat vir besproeiingsdoeleindes gebruik word, te ontsmet om uiteindelik die risiko van kontaminasie vir die verbruikers van vars produkte te verminder.

Monsters is vyf keer tussen die nat winter- en droë somerseisoene uit elkeen van vier riviere in die Wes-Kaap geneem om die mikrobiese en fisies-chemiese profiele van die riviere vas te stel. Hierdie resultate is vergelyk met die riglyn-limiete. Die monsters is op laboratoriumskaal aan drie dosisse (20, 40 en 60 mJ.cm^{-2}) LP UV-bestraling blootgestel. Daar is vasgestel dat LP UV-bestraling effektief was om die mikrobiese lading tot nie-waarneembare vlakke te verminder. Patogene mikroörganismes is suksesvol geïnaktiveer na 'n dosis van 20 mJ.cm^{-2}. Die heterotrofiese kolonie-getalle het meer geleidelik verlaag en het dus groter weerstand teen behandeling getoon. Dertien isolate is verkry en geberg vir toekomstige eksperimente. Daar is voorgestel dat 'n voorafbehandelingsstap geïmplementeer word om die fisiese kwaliteit van die rivierwater te verbeter voor UV behandeling.

Isolate van *E.c oli* (n = 3), *Salmonella* species (n = 2) en *L. monocytogenes* (n = 8) is gestoor vir latere toestsing. Die gebergde *L. monocytogenes*-isolate is onderwerp aan stam tiperingseksperimente, waar vasgestel is dat al die isolate aan stam I behoort. Hierdie stam word meestal met listeriose geassosieer. Uitgebreide-spektrum beta-laktamase (ESBL) toetse het aangedui dat geen van die *Enterobacteriaceae*-isolate ESBL-produseerders was nie. Alle *Enterobacteriaceae*-isolate (n = 5) het weerstand teen tetrasiklien, ampisillien en trimetopriem-sulfametoksasool getoon. Weerstand van *L. monocytogenes* isolate (n = 5) is waargeneem teen trimetopriem-sulfametoksasool, terwyl vier *L. monocytogenes* isolate weerstand getoon het teen ampisillien, penisillien en eritromisien. Veelvuldige middelweerstand is gerapporteer vir 90% van die rivierwater-isolate (n = 9).

Vier verskillende sakfilter poriegroottes (5, 20, 50 en 100 µm) is ondersoek om die mees effektiewe voorbehandelingstap te bepaal om die UV-transmissie (UVT %) van die water te verbeter. Hierdie eksperiment is uitgevoer op die ‘slegste geval scenario'-rivier, die Mosselbankrivier. Verbeterings in die totale gesuspendeerde vastestofinhoud, chemiese suurstofbehoefte en troebelheid is gerapporteer, maar die uiters hoë totale opgeloste vastestofinhoud (728.67 mg L^{-1}) het 'n groter verbetering in die UVT % verhoed. Daar is vasgestel dat die 5 µm sakfilter die doeltreffendste poriegrootte was.

In die huidige studie kon LP UV-bestraling suksesvol aangewend word om water van 'n aanvaarbare standaard vir die besproeiing van vars produkte te produseer. Die fisiese kwaliteit van die water het nie 'n suksesvolle ontsmetting verhoed nie, maar het die blootstellingstyd wat nodig was om 'n spesifieke dosis te lewer, verhoog en dus die doeltreffendheid verminder. Daar is vasgestel dat LP UV-bestraling patogene mikroörganismes kan verminder tot nie-waarneembare vlakke. Hierdie ontsmettingsmetode toon dus belofte vir volskaalse toepassing op besproeiingswater.

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This thesis is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of three research chapters, which is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Contents

ABBREVIATIONS

AOP	Advanced Oxidation Process
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
BOD	Biological Oxygen Demand
BHI	Brain Heart Infusion Agar
BPW	Buffered Peptone Water
COD	Chemical Oxygen Demand
CDC	Centre of Disease Control
CFU	Colony Forming Units
CLSI	Clinical & Laboratory Standards Institute
CPD	Cyclobutane Pyrimidine Dimers
CV	Clavulanic Acid
CP	Cefepime
CTX	Cefotaxime
CAZ	Ceftazidime
DAEC	Diffusely Adherent *Escherichia coli*
DBPs	Disinfection By-products
DNA	Deoxyribonucleic acid
dNTP	Deoxy ribonucleotide triphosphate
DWAF	Department of Water Affairs and Forestry
E. coli	*Escherichia coli*
EAEC	Enteroadhesive *Escherichia coli*
EC	Electrical Conductivity
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
ESBL	Extended-Spectrum Beta-Lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAD	Flavin Adenine Dinucleotide
FAO	Food and Agricultural Organisation
FDA-BAM	Food and Drug Administration Bacteriological Analytical Manual
FR	Franschhoek River
HIV/Aids	Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
HPC	Heterotrophic Plate Count
HUS	Haemolytic-uremic Syndrome
ICU	Intensive Care Unit
ISO	International Organization for Standardization
kGy	Kilo Gray

L-EMB	Levine Eosin-Methylene Blue Agar
LP	Low-pressure
MALDI-TOF	Matrix-assisted Laser Desorption/Ionization Time-of-Flight
MDR	Multidrug Resistant
MP	Medium-pressure
MPF	Minimally Processed Foods
MR	Mosselbank River
NA	Nutrient Agar
NER	Nucleotide Excision Repair
NTU	Nephelometric Turbidity Units
OD	Optical Density
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
PR	Plankenburg River
R	Resistant
RTE	Ready-to-Eat
S	Susceptible
SA	South Africa
SABS	South African Bureau of Standards
spp.	Species
SANS	South African National Standards
STEC	Shiga-toxin producing *Escherichia coli*
TB	Tuberculosis
TDS	Total Dissolved Solids
TSB	Tryptic Soy Broth
TSS	Total Suspended Solids
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UV-G	Germicidal Ultraviolet
UVT %	Ultraviolet Transmission Percentage
VRBG	Violet Red Bile Glucose Agar
V-UV	Vacuum-UV
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate agar

Chapter 1

INTRODUCTION

Water is a natural resource that is indispensable for the production of food. Approximately 63% of the available fresh water is used for agricultural purposes in South Africa (Donnenfeld *et al.,* 2018). However, population and economic growth continue to place immense pressure on the fresh water availability, limiting the quantity available for the agricultural irrigation of fresh produce (Hanjra & Qureshi, 2010). In South Africa, surface water, which includes rivers, dams and lakes, is the preferred source for agricultural irrigation due to the cost and ease of usage (Singh, 2013, Maree *et al.,* 2016). Maree *et al.* (2016) indicate that of the total available fresh water in South Africa, the surface water usage totals 77%. Zhou *et al.* (2012) explains that rivers and other surface waters are frequent recipients of contaminants from the surroundings, which results in a land-water interaction. Based on the type and extent of the contaminant, the resulting water may have a negative impact on the functions that it is required for. According to Donnenfeld *et al.* (2018), over 60% of rivers in South Africa are currently overexploited. Apart from the concerns regarding water availability, concerns regarding water safety and quality have increased dramatically in South Africa (Britz *et al.,* 2013).

It has been reported that microorganism carry-over from irrigation water to crop is a major concern in the case of food safety and can result in foodborne disease outbreaks (Zimmer-Thomas & Slawson, 2007, Huisamen, 2012). Pachepsky *et al.* (2011) has indicated that irrigation water is a major pre-harvest contributor to the contamination of fresh produce. Other environmental sources of contamination include faecal contamination, pesticides and other chemicals and contaminated soil (Olaimat & Holley, 2012). Pathogens that have been commonly associated with fresh produce include pathogenic strains of *Escherichia coli (E. coli), Listeria monocytogenes, Salmonella* species (spp.), viruses and parasites (Jung *et al.,* 2014). Fresh produce-related outbreaks have increased in the last two decades, which has been observed globally (Herman *et al.,* 2015). In South Africa, limited reporting of food-related outbreaks exists, which is due to the lack of a surveillance reporting system (Laubscher, 2019). Painter *et al.* (2013) reported that 46% of all foodborne outbreaks in the United States from 1998 to 2008 were traced back to produce-associated illnesses. The push towards a healthier diet that comprises of fresh fruit and vegetables is contradicted by the threat of produce contamination with pathogens.

The Department of Water Affairs and Forestry (DWAF) stipulates the limits for microbial loads in irrigation water, as well as other physical characteristics (DWAF, 1996). This has since been updated by du Plessis *et al.* (2017) with the Water Research Commission in the

form of the Decision Support System for risk-based and site-specific guidelines for irrigation water. The limit for faecal coliforms is 1 000 colony forming units (cfu) per 100 mL in water intended for the irrigation of fresh produce. Studies performed by multiple researchers in the Western Cape (Barnes & Taylor, 2004, Paulse *et al.,* 2009, Lamprecht *et al.,* 2014, Olivier, 2015, Sivhute, 2019) have indicated that microbial contamination in river water continuously exceeded the guideline limits. There is no stipulated guideline in South Africa, nor many other countries around the world, for the presence of pathogens such as *Salmonella* spp. or *L. monocytogenes* in irrigation water. This may result in underreporting, as there is no legislative pressure to test for these organisms.

E. coli is frequently used as an indicator organism of faecal pollution in water, and this is, therefore, used as a method of quantifying the level of pollution in the river water (Britz *et al.,* 2012). Due to the constant presence of high faecal coliform contamination, consistent monitoring of the microbial levels in rivers used for irrigation has been performed in South Africa, and more locally – in the Western Cape. The Plankenburg, Eerste, Mosselbank and Krom Rivers have been analysed in a number of studies over the last decade (Lötter, 2010, Huisamen, 2012, Olivier, 2015, Sivhute, 2019). Lötter (2010) reported faecal coliform levels of 1.6 x 10^5 and 4.6 x 10^5 cfu. 100 mL^{-1} in the Plankenburg and Mosselbank Rivers, respectively. Huisamen (2012) reported findings of up to 7 x 10^6 *E. coli* cfu. 100 mL^{-1} in the Plankenburg and Eerste Rivers. In 2016, Alegbeleye *et al.* investigated the microbial loads in the Plankenburg and Eerste Rivers. It was reported that average bacterial counts in the Plankenburg River ranged between 3.1 x 10^5 to 6.9 x 10^8 cfu.mL^{-1}. More recently, Sivhute (2019) noted *E. coli* levels of over 3.1 x 10^6 cfu. 100 mL^{-1} in the Plankenburg River. Another concerning issue is the ability of microorganisms to acquire resistance to antimicrobials, as well as the presence of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae*. Both ESBL-producers and antimicrobial resistant bacteria have frequently been identified within surface waters around the world (Blaak *et al.,* 2015, Vital *et al.,* 2018). Limited research has been recorded with regard to the persistent presence of antimicrobial resistance genes in South Africa. However, the isolation of antimicrobial resistant bacteria from river water isolates has increased in research (Romanis, 2013, Lamprecht *et al.,* 2014, Laubscher, 2019, Sivhute, 2019, Richter *et al.,* 2019). These results provide an indication of the consistent contamination of the rivers and emphasize the need for an effective method of disinfection. Sigge *et al.* (2016) suggested that the ultimate solution for the contaminated river water problem is treating the pollution at the source, or better yet, prevention of the pollution itself. Based on water quality studies of local rivers, Britz *et al.* (2012) has suggested that treatment strategies that result in a target microbial reduction of

3 – 4 log units should be sufficient to result in water with *E. coli* loads that fall within the guideline limits.

Water disinfection includes physical, chemical and photochemical methods (National Health and Medical Research Council (NHMRC), 2004). Lavonen *et al.* (2013) & Olivier (2015) states that the efficacy of these methods of water treatment is dependent on the water quality, which is highly variable in surface waters. The oldest method of water disinfection is the use of filtration techniques, where particulates are physically removed from the water (Kesari *et al.,* 2011). Chlorine, peracetic acid and hydrogen peroxide are commonly used chemicals for water disinfection (Jyoti & Pandit, 2004). These chemicals are associated with the development of carcinogenic disinfection by-products (DBPs), particularly in the case of chlorine. This has resulted in a push towards environmentally friendly methods of water disinfection (Galvéz & Rodrígues, 2010).

Ultraviolet (UV) radiation has gained momentum as a method of disinfection due to the reduced environmental impact, no residual chemicals, and efficacy of water treatment (Liu *et al.,* 2005, Guo *et al.,* 2009). Bolton & Cotton (2008) state that UV radiation is effective at disinfecting pathogens such as *Cryptosporidium* and *Giardia* spp. which are organisms that are known to be resistant to chlorine disinfection. The nucleic acids of the microorganisms absorb the UV radiation, predominantly in the region of 253.7 nm, which results in the formation of either cyclobutane pyrimidine dimers (CPDs) or pyrimidine 6-4 pyrimidones (6-4PPs) (Dai *et al.,* 2012, Cutler & Zimmerman, 2011). This process results in the prevention of transcription, resulting in mutagenesis, and ultimately leads to cell death (Cutler & Zimmerman, 2011, Gayán *et al.,* 2012).

Either low-pressure (LP) or medium-pressure (MP) mercury vapour lamps are utilised to apply UV treatments (Bolton & Cotton, 2008). Most commonly seen in literature, LP lamps and laboratory-cultured strains are utilised to determine dosage requirements of microorganisms, particularly *E. coli*. This has resulted in a number of conflicting reports with regard to UV dosage requirements, particularly for environmental or clinical isolates that show greater resistance to disinfection than pure, laboratory-cultured organisms (Maya *et al.,* 2003).

Dosage requirements are dependent on a number of factors, which include both intrinsic and extrinsic characteristics (Gayán *et al*., 2014). Intrinsic characteristics include factors such as cell size, presence of UV absorbing proteins, cell wall thickness and repair mechanisms, amongst others (Koutchma, 2009). Extrinsic characteristics include the physical and chemical properties of the water, such as the UV transmission percentage (UVT %), turbidity and suspended solids, amongst others (Olivier, 2015, Farrell *et al*., 2018). Inevitably, a great deal

of variation exists with regard to microbial resistance or sensitivity towards UV disinfection, and needs to be taken into account when determining dosage requirements (Liu, 2005).

Overall, this method of water treatment has shown to be effective for producing a safe supply of water for the irrigation of fresh produce, as noted in previous studies (Hassen *et al.,* 2000, Jones *et al.,* 2014, Sivhute, 2019). Several factors need to be taken into account for ensuring a consistent water disinfection, which includes the variabilities in river water quality, microbial loads present and type of UV radiation equipment employed.

Findings from previous research (Olivier, 2015 & Sivhute, 2019) indicated that the physico-chemical characteristics of the water sample may impact the UV disinfection efficacy, as well as increasing the exposure time required for disinfection. These studies were limited to water sampled from rivers with relatively similar physical profiles. It was, thus, recommended that the impact of the physico-chemical profile on UV treatment is studied across a broader range of river water sources. Another recommendation from these research studies included the implementation of a filtration step to improve the physical characteristics of the water prior to UV radiation.

Previous studies have investigated the effect of UV radiation on *E. coli strains* (Olivier, 2015 & Sivhute, 2019, Mofidi *et al.,* 2002, Zimmer-Thomas *et al.,* 2007). No research has, however, been performed in South Africa, with regard to the application of UV radiation on environmental strains of other food pathogens such as *Salmonella* spp. and *L. monocytogenes*, resulting in a literature gap in this area. It was, thus, recommended that the effects of UV treatment on pathogens such as *Salmonella* spp. or *L. monocytogenes,* are also investigated (Olivier, 2015 & Sivhute, 2019).

The greater aim of the current research study was to evaluate the efficacy of LP UV radiation on four different river water sources that are used for agricultural irrigation, to determine the effect of varying water quality on UV disinfection at laboratory-scale. This study investigated the effect of UV on the food pathogens, *L. monocytogenes* and *Salmonella* spp. in order to determine the dosage requirements for the inactivation of these organisms. Resistance profiles of isolates obtained from the rivers were tested, including antimicrobial resistance testing. Lastly, the transition between LP laboratory-scale UV radiation to pilot-scale MP UV radiation was initiated through the evaluation of a pre-treatment step to improve the physico-chemical characteristics of the river water prior to treatment. By filling these knowledge gaps, this study intends to contribute towards the successful future application of UV radiation in irrigation water treatment at farm-scale.

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Chapter 2

LITERATURE REVIEW

2.1 Introduction

“When the well is dry, we know the worth of water” - Benjamin Franklin.

Although this statement was made in 1746, it still seems as if the essentiality of water is not fully understood. Agricultural and industrial practices, and human and animal survival are all dependent on the amount and quality of water that is available for use. Water quality is defined as the biological, chemical and physical characteristics of the water (Bhagwan, 2008). Food security, according to Hanjra & Qureshi (2010), is under threat as water demands, as a result of rapid increases in urbanisation and industrialisation, continue to overshadow demands for water used for irrigation purposes.

The state of water in South Africa is either described as “too little”, as a result of drought conditions or over-usage or “too dirty”, as a result of pollution (Singh, 2013, Maree *et al.,* 2016). Microbiological studies regarding the water quality in rivers around the Western Cape have proven that the water is not fit for irrigation without pre-treatment (Huisamen, 2012, Britz, 2012, Britz, 2013, Omar & Barnard, 2010). Surface waters that are contaminated with pathogenic microorganisms might result in widespread outbreaks of diarrhoeal infections, causing developmental disabilities in children that could easily be preventable by correct water treatment facilities and disinfection practices (World Health Organisation (WHO), 2014). Surface waters pose a greater risk for contamination, but is often the first choice for irrigation purposes as it of greater economic feasibility to use than groundwater (Singh, 2013, Maree *et al.,* 2016). Britz *et al.* (2013) states that a diet that contains fresh produce could prevent illnesses such as cardiovascular diseases. Contradictorily, fresh produce has been linked to being carriers of pathogens that result in foodborne outbreaks and oftentimes this is connected to the irrigation water quality (Britz *et al.,* 2013, Uyttendaele *et al.,* 2015).

Water treatment methods such as chemical, physical and photochemical processes can alleviate these pathogenic risks and have been employed worldwide. These methods work by lowering, deactivating or removing organisms that can result in health risks for consumers (National Health and Medical Research Council (NHMRC), 2004).

2.2 Adverse environmental conditions affecting water quality

South Africa is dominated by a semi-arid climate, however, rainfall patterns can vary between 100 mm per annum to over 1500 mm per annum between the eastern and western sides of the country respectively, with a yearly average of around 450 mm (Chami & Moujabber, 2016). South Africa has been described as the 29th driest country out of 193 countries in terms of

"Total Actual Renewable Water Resources" (TARWR) (Blignaut & Van Heerden, 2009). The year 2015 has been reported as the driest year in data collected since 1904, as well as being unprecedentedly hot with temperatures rising approximately 3.4°C on average for the year. This led to the establishment of "Day Zero" in the city of Cape Town, where fresh water was predicted to run out in April 2018 even after massive water rationing was implemented (Masante *et al.,* 2018). The Western Cape is described as having a Mediterranean climate, where the province has wet winters and dry summers as well as exhibiting a semi-arid climate towards the interior. Between 2015 and 2018, it was noted that an area in Cape Town received 35% less than the expected rainfall for that area during that period. Within July of 2017, the wettest month in this time-frame received less than half of the normal precipitation predictions for this month (Masante *et al.,* 2018). As of early January 2018, water levels within the Western Cape dams that have the capacity to store nearly 900 000 mega-litres (ML) of water were cumulatively at 26.9 % of capacity, with 241 358 ML of water (Masante *et al.,* 2018). Masante *et al.* (2018) describe that deficits of between 70% and 80% can be noted every 10 years in the Western Cape as well as a major increase in the frequency of heatwaves occurring in the last 10 years resulting in negative impacts on human health as well as socio-economic activities (Masante *et al.,* 2018).

Droughts not only reduce the amount of water available for daily tasks, but affects the quality of water as well. Salinity has been shown to increase in streams and rivers during drought periods and can be attributed to evapo-concentration as well as a decrease in dilution of highly saline groundwater systems (Mosley, 2015). Reductions in nutrient content during droughts have been shown in rivers and streams as a result of reduction of catchment inputs, increased uptake of dissolved nutrients by algae and macrophytes or longer water residence times resulting in an increase in denitrification (Mosley, 2015). Donnelly *et al.* (1997) showed that toxic cyanobacterial species, such as *Anabaena circinalis,* bloomed extensively in a drought-period which was characterised by low river flow and phosphorous release from the anoxic sulphate-reducing sediments within a river in Australia. Smith *et al.* (2015) noted a two- to three-fold increase in *Cryptosporidium* oocysts and *Giardia* cysts in fresh water samples in periods of extreme weather conditions, which includes both flood and drought conditions, as compared to that of normal conditions. Indirect impacts of extreme weather conditions as well as changing trends are often overlooked as they take months or years to present themselves after the particular weather event occurred. These impacts can be identified in the form of wildfires, the encouragement of the growth of invasive species and increased forest mortality (Khan *et al.,* 2015). Droughts in areas of Australia and the USA have led to water quality reductions as a result of increased turbidity, compounds affecting taste and odour, and disinfection by-products (Mosley, 2015). As a drought will naturally decrease surface water

levels, it too will reduce the amount of groundwater available due to increased pumping or lower recharge rates (Fig. 1). This results in the quality dramatically decreasing due to intrusion of poor quality groundwater or seawater in the case of coastal areas. This seawater increases the bromide concentrations within the freshwater, which causes toxic disinfection by-products to form (Kahn *et al.,* 2015). Drought conditions, often associated with extreme heat cause bodies of surface water to increase in temperature. These water temperature increases, according to Lutz *et al.* (2013), are positively correlated to the presence of *Vibrio cholerae,* especially in surface waters above 15°C. Other than *V. cholerae,* high water temperatures have been associated with the proliferation of pathogenic bacterial strains. Importantly, every 5°C increase in water temperature results in chlorine residual decay at double the rate, reducing the residual disinfection capacity and having potentially devastating effects on water quality (Fisher & Knutti, 2015).

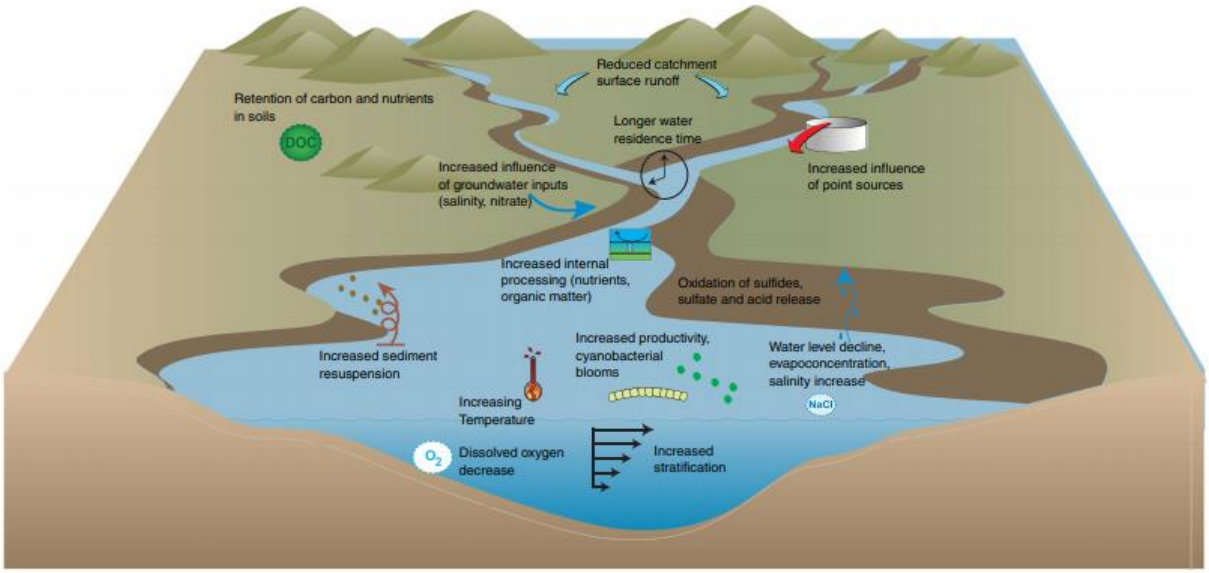

Figure 1 The effect of drought conditions on chemical, physico-chemical as well as microbial characteristics on water (Mosley, 2015)

Heavy rainfall as well as flooding has shown too to decrease water quality in both surface and groundwater systems. Interestingly, flood periods are associated with an increase in Ultraviolet (UV) absorbing compounds, such as aromatic compounds, thereby, reducing the efficacy of UV treatment (Kahn *et al.,* 2015). Flooding conditions increase both turbidity as well as dissolved organic matter within water samples, which require additional treatments to reduce to the turbidity and organic matter to acceptable levels (Göransson *et al.,* 2013). Sewer overflow, as a result of heavy rainfall, can cause catastrophic effects with regard to microbial water quality (Kahn *et al.,* 2015).

Limited research has been conducted in South Africa regarding the direct microbial quality and water quality changes during a drought period. Dearmont *et al.* (1998) states that

a 1% increase in turbidity results in chemical costs increasing by 0.25% per litre. Athar & Ahmad (2002) describe that an increase in the metal content in water, as a result of contamination from mining, results in lower plant crop growth rates by between 13% and 70% but also decrease the yield of wheat by up to 83%. An increase in salinity, to a level of 1 200 $mg.L^{-1}$, which was noted in the Middle Vaal River in South Africa, can have direct costs of up to R183 million per annum (Nieuwoudt *et al.,* 2004). Water quality reduction as a direct result of human impact can be attributed to a number of factors. The most notable in South Africa being that of faecal contamination from poor sanitation in rural areas and informal settlements, agricultural run-off (fertilisers and pesticides) and acid mine drainage (Colvin *et al.,* 2016).

2.3 Current state of fresh water supplies and future requirements in South Africa

A report by Schreiner *et al.* (2018) describes the water requirements per sector in South Africa. Agriculture, the sector that places the greatest demand on water in the country, requires approximately 60% of fresh water. Other sectors placing pressure on the fresh water supply include the municipal sector (27%), power generation (4.3%) and mining and industrial demands requiring 3.3% and 3%, respectively (Schreiner *et al.,* 2018). The report continues to explain that 7% of formal employment in South Africa is from the agricultural sector, which directly or indirectly impacts 8.5 million individuals. The agricultural industry contributes to 3% of the national GDP.

From the total amount of rainfall received in South Africa per annum, only 9% reaches rivers and surface waters and 4% recharges the groundwater supplies (Colvin *et al.,* 2016). Colvin *et al.* (2016) states South Africa was one of the first countries in the world to implement water allocations per capita, which has allowed the maintenance of a sustainable water supply. Periods of drought in recent years placed incredible pressure on the fresh water supply and resulted in water restrictions of 50 litres per person per day to prevent depletion. South African water supplies currently provide 235 litres of fresh water per capita per day, whereas the global average currently allows for 175 litres per capita per day (Donnenfeld *et al.,* 2018). Furthermore, it has been estimated that 60% of rivers in South Africa are currently being overexploited, where only 33% of rivers can be considered to be in a good condition (Donnenfeld *et al.,* 2018). Population growth in South Africa in the next 10 years will result in a predicted 32% increase in fresh water demand in the country, with a population increase of 3.3 million individuals (Donnenfeld *et al.,* 2018). As of April 2017, over 5.3 million households did not have access to a reliable and safe water supply in South Africa (DWAF, 2019). Data shows that municipal requirements are predicted to increase from 27.4% to 31.5% by the year 2035, attributed to the predicted population increase as well as the rapid urbanisation of the population. South Africa's fresh water withdrawals for 2017 can be seen in Fig. 2 as compared

to the predicted withdrawals for 2035, where agricultural demands remains responsible for the largest usage per sector.

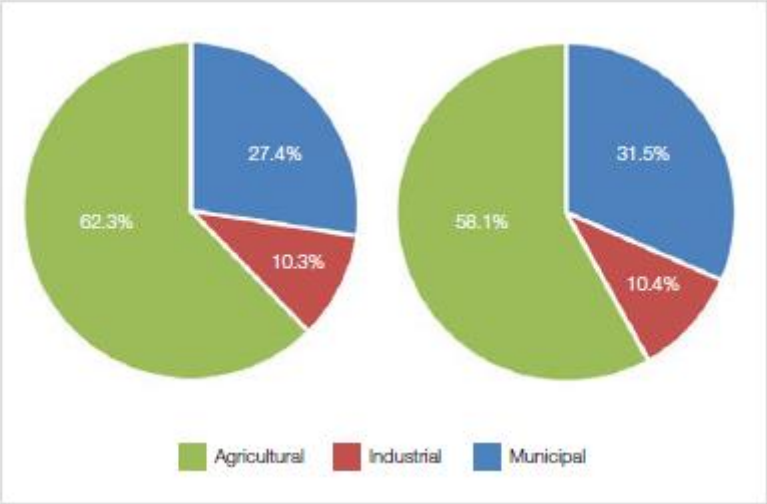

Figure 2 Water demands per sector for South Africa in 2017 (left) and predicted withdrawals for 2035 (right) (Donnenfeld *et al.*, 2018)

A current projection states that South Africa will face a 17% water deficit by 2030 (DWAF, 2019). This has led to research into the development of a Master Plan by the Department of Water Affairs and Forestry (DWAF) which aims to build a secure water future in South Africa. Ground water usage currently supplies only 15% of the fresh water in South Africa (Colvin *et al.,* 2016). This National Water and Sanitation Master Plan (DWAF, 2019) aims to increase the ground water usage, which is greatly limited by the geology of the country, as well as the implementation of desalination processes. A decrease in the demand on unreliable surface water and to reduce consumer demand on fresh water from 235 litres to 175 litres per capita per day by 2040, are but a few of the methods that will be employed to alleviate the water deficit issue (Britz *et al.,* 2012, DWAF, 2019). In South Africa, poor water quality standards have been attributed to poorly maintained infrastructure and equipment in treatment facilities. Reasons include faulty operating procedures, lack of routine maintenance and operator errors (Council for Scientific and Industrial Research (CSRI), 2007).

2.3.1 Western Cape Rivers and their Microbiological State

According to Sousa *et al.* (2007), surface waters are unpredictable in microbial loads and physico-chemical characteristics. This could be attributed to the variations in climate and seasonal changes as well as upstream commercial or recreational activities resulting in contaminants flowing into the water source (Sousa *et al.,* 2007). Numerous studies have been performed at Stellenbosch University over the last nine years, with conclusions that indicate that the water quality of the rivers used for irrigation in the Western Cape are of an unacceptable standard, and place great risk for carry-over of microorganisms from

contaminated water to fresh produce (Lötter, 2010, Huisamen, 2012, Olivier, 2015, Giddey, 2015, Bester, 2015, Van Rooyen, 2018, Sivhute, 2019). Barnes (2003) determined the Plankenburg River quality over various months of the year, at four sampling points over a period of five years. Dramatic increases in faecal coliform counts were noted in water samples withdrawn before Kayamandi informal settlement and after it (from 329 cfu. 100 mL^{-1} to 4.93 x 10^7 cfu. 100 mL^{-1}, respectively). Lower increases were observed in the winter months, attributed to lower river temperatures as well as increased rainfall resulting in the dilution of the microbial load. A baseline study determined the presence of indicator and index microorganisms in the Plankenburg and Eerste Rivers in the Western Cape, and the results indicated that the presence of faecal indicators reached 7 log cfu. 100 mL^{-1} (Britz *et al.,* 2012). Huisamen (2012) noted a colony count of between 310 to 7 x 10^6 cfu. 100 mL^{-1} for faecal coliforms in the Plankenburg River in the same year. Western Cape river microbial counts observed in previously mentioned studies dramatically exceeded the guidelines indicated by the Water Quality Guidelines (DWAF, 1996a). Rivers tested in the Western Cape were contaminated, not only with faecal coliforms such as *E. coli*, but pathogens capable of causing widespread water- or foodborne outbreaks such as *L. monocytogenes* and *Salmonella* species (spp.) were identified as well (Huisamen, 2012). These high microbial loads resulted in the water being classified as unacceptable for irrigation without treatment.

According to Zimmer & Slawson (2002) and Rodrigues *et al.* (2020), the usage of contaminated river water for the irrigation of fresh produce has been linked to an increasing amount of foodborne outbreaks. The risk of becoming infected from fresh produce and the quantity of contaminated produce consumed has shown to have a positive correlation (Britz *et al.,* 2012).

2.4 Irrigated produce resulting in foodborne outbreaks

The consumption of fresh produce has increased globally in the last three decades due to the advent of new technologies, providing consumers with the convenience and ease of opening a pre-washed bagged salad or freshly cut fruit. Predictions of fresh produce market demands in South and East Africa are expected to quadruple by the year 2040, and the total market size for perishable foods is set to increase eight-fold in the same time period (Tschirley *et al.,* 2014, Grace, 2015). Ironically, consumer consumption of fresh produce such as fruit and vegetables forms part of a healthy lifestyle, however, contamination of fresh produce with pathogens has led to increasing public health concerns over the past two decades. This is attributed to the fact that fresh produce is not processed further than initial washing, in any way that is able to eliminate pathogens effectively (Jung *et al.,* 2014). Jung *et al.* (2014) describes that most likely sources of contamination of fresh produce is attributed to irrigation

water, soil, field workers, processing plants and retail handling which have all proven to compromise the safety of the food product in some way. Hsu *et al.* (2006) & Jung *et al.* (2014) denote that the growth of pathogens such as *L. monocytogenes, E. coli* O157:H7 and *Salmonella* spp. can be controlled by maintaining a consistent cold chain at refrigerated temperatures, however, this method is insufficient to ensure complete consumer safety. Widespread foodborne outbreaks of salmonellosis and pathogenic strains causing *E. coli*-related infections have routinely been associated with fresh produce worldwide. In 2016, 720 individuals across the United States became infected with *Salmonella poona* which was linked to cucumbers grown in Mexico. This outbreak resulted in 204 hospitalisations and six deaths (United States Food and Drug Administration (U.S. FDA), 2016). Investigations into the cause of outbreak led to wastewater management concerns as well as the design of the cucumber pre-wash area (United States Food and Drug Administration (U.S. FDA), 2016). The outbreak with the highest death-toll was caused by *E. coli* in 2011, where an O104:H4 outbreak related to fenugreek sprouts affected 4000 individuals. This resulted in 850 cases of haemolytic uremic syndrome (HUS) and claimed the lives of 54 individuals (Frank *et al.,* 2011). Further investigations suggested that contamination occurred during the sprouting stage, most likely due to the water quality (Frank *et al.,* 2011). Michino *et al.* (1999) reported on the largest *E. coli* O157:H7 outbreak ever recorded, in which 12 000 cases were reported and lead to 12 deaths. This outbreak was related to raw radish sprouts in Japan (Michino *et al.,* 1999). A listeriosis outbreak in the United States in 2011 resulted in 31 deaths as a result of cantaloupe that was contaminated with *L. monocytogenes* (Centre for Disease Control (CDC), 2011). Between the years of 1973 and 2010, almost 2000 cases of salmonellosis were reported across the United States, which resulted in three deaths. This was as a result of *Salmonella enterica* infection from tomatoes (Bennett *et al.,* 2014). Trace-back investigations showed that a majority of the outbreaks were as a result of farm-level contamination (Bennett *et al.,* 2014). A study that took place in the Eastern Cape in 2012 investigated the prevalence of foodborne pathogens in ready-to-eat foods found in roadside cafeterias. Nyenje *et al.* (2012) describes that these roadside cafeterias provide food security for low-income urban workers as well as the livelihood of individuals in developing countries. The results of this investigation showed that of the investigated food products which included vegetables, rice, pies and meat stews; vegetables had the highest microbial counts, specifically *Enterobacteriaceae*. These counts were as high as 6.8 log which equals over 6 million cfu.g^{-1} (Nyenje *et al.,* 2012). These high counts, were associated with the irrigation water quality used during crop watering, lack of running water in the cafeteria, refrigerators, lack of hygiene from the food handlers and wash buckets filled with unsanitary water that are used to clean utensils and equipment (Nyenje *et al.,* 2012). Statistics provided by the WHO (2008) indicate that 1.4 million child deaths worldwide are as a result of diarrhoea, 860 000 child deaths due to malnutrition and two billion

intestinal nematode infections could be entirely preventable through adequate sanitation and reinforced hygienic practices. Water quality, used for drinking or crop irrigation is, therefore, of utmost importance to ensure consumer safety.

2.5 Indicator organisms used as a measure of water quality and food

Shtawa (2016) states that the concern over the microbiological quality of water available for both irrigation and domestic purposes is ever-growing, with a staggering one-third of intestinal infections worldwide being as a result of waterborne diseases. Furthermore, 40% of all diarrhoea-related deaths worldwide are as a result of poor sanitation, hygiene and water quality (Shtawa, 2016). Contamination of irrigation water can occur as a result of numerous factors, including animals defecating in the rivers or individuals in rural areas with limited access to proper toilet facilities using bushes close to the rivers as toilet areas which ends up in rivers after rainfall. These faecal coliforms, from both humans and animals, now in the rivers used for irrigation purposes, are then passed onto crops often without further treatment. This is of major concern as many rural households and subsistence farmers are dependent on minimally processed foods (MPF) as their major daily intake of food. These foods are not processed with any chemicals or heat treatment before consumption, resulting in the consumption of contaminated fruit and vegetables and possibly leading to illnesses (Britz *et al*., 2012). In some water-scarce countries, the use of grey and domestic wastewater which often includes human sewage, is utilised as irrigation water to reduce the requirement on fresh, clean water for irrigation purposes. Incorrect handling and treatment of this water can result in extremely high microbial loads contaminating food and water sources (Steele & Odumeru, 2004).

The term “indicator organism” is one that is used to describe organisms whose presence or absence describes a specific feature of concern, therefore, one that is used to determine microbiological criteria for food safety, and suggests a possible microbial hazard or pathogen (Forsythe, 2010). The criteria are used to ensure product quality, safety, hygiene and a possible prediction of shelf-life (Montville *et al.,* 2012a). According to Forsythe (2010), the criteria to be considered an indicator organism state that it should be one that is easy to detect; be present when the concerning pathogen is present as well as having the same growth rates and requirements for survival as the pathogen; amongst others. Many indicator organisms exist for contamination in different kinds of food sources, which are primarily food spoilage organisms. Savichtcheva & Okabe (2006) describe total and faecal coliforms as well as *E. coli* as being indicators of faecal contamination that could indicate the possible presence of enteric pathogens. This is in contrast to an index organism which is used to describe the behaviour as well as the presence of a particular organism in an environment, noting that an organism can be both an indicator and an index organism (McEgan *et al.,* 2013). The presence of an

index organism can be used to indicate the probability of a pathogen in a sample, for example, the presence of *E. coli* in a water sample may be an indication of *Salmonella* spp. presence. This, however, has its limitations as the pathogen may not necessarily be present even when it is assumed to be. The tests for the index organisms are generally simpler and cheaper to conduct as compared to the test for the pathogen (McEgan *et al.,* 2013).

It therefore, can be noted that the presence of faecal coliforms can be an effective indication of poor water quality, due to their ability to survive in ubiquitous environments, and not limited to organisms present in the gut of warm-blooded animals. However, not all strains can impart negative characteristics on human or animal health. The advantage of testing for Enterococci spp. as opposed to *E. coli* is that these organisms are able to remain alive for longer than *E. coli* and therefore will prevent the chance of obtaining false negatives when testing (Wiley *et al.,* 2014).

In the U.S., the test for faecal contamination was historically determined by the presence of total coliforms. The European Union (EU) has increased the testing for Enterococci spp. as an indicator of faecal contamination. After countless outbreaks worldwide and the need for uniformity with regard to testing methods for contamination, Boehm and Sassoubre (2014), state that the US, EU and WHO have collaborated in adopting the test for Enterococci spp. as an indicator of contamination and water quality for water used recreationally and drinking water as it allows for greater specificity. However, testing for *E. coli* remains an acceptable test for faecal contamination (Ricci *et al.,* 2017).

2.5.1 Enterobacteriaceae family

Enterobacteriaceae is a large family of approximately 20 genera that are genetically and biologically similar, including both pathogenic and non-pathogenic organisms. These organisms can be found in a variety of environments. The physiological diversity of this large family proves difficult to provide specific characteristics for survival, however, several intrinsic parameters have been indicated by Baylis *et al.* (2011). *Enterobacteriaceae* can be classified as being psychrotrophic or mesophilic, with water activity requirements being limited to 0.95 (Baylis *et al.,* 2011). The term water activity describes availability of water in the food, indicating the amount of water that is not bound or immobilised by surrounding particles. Therefore, manipulating the water activity of a food product can be an effective measure of inactivating these microorganisms (Montville *et al.,* 2012a). The wide pH range, pH 3.8 – 9.0, that has been indicated for the survival of *Enterobacteriaceae* can be attributed to the variety in environmental demands for survival of a diverse family. Facultative anaerobes are able to grow both on the surface as well as the interior of foods, without being inhibited by the growth of strict aerobes. It is interesting to note that the proliferation of *Enterobacteriaceae* can inhibit

the growth of aerobic spoilage microorganisms (Baylis *et al.,* 2011).

Enterobacteriaceae rely on the fermentation of glucose for survival, with a few exceptions, such as *Aeromonas* spp. and *E. coli* being able to ferment both glucose and lactose (Baylis *et al.,* 2011). Within the *Enterobacteriaceae* family resides the group coliforms. Historically, coliforms were the primary test performed to determine whether contamination by faecal matter has occurred, however, it has been noted that some coliforms are found in other environments, such as plants (Odonkor & Ampofo, 2013). This indicates that faecal contamination might not have occurred if a positive test for coliforms has been noted. Coliforms have no specific taxonomic grouping, however, can be described as showing β-galactosidase activity when chromogenic media, such as violet red bile glucose agar, is used, as well as producing acid and gas by traditional testing methods.

2.5.2 E. coli *pathogenesis, characteristics and its presence within water sources*

E. coli are Gram-negative, catalase-positive and oxidase-negative rod-shaped organisms that are an integral component in the functioning of the intestine of humans and animals, forms part of the facultative anaerobic flora, as well as being incapable of forming spores (Levine, 1987). Shtawa (2016) explains that *E. coli* can be considered to be a more specific indication of faecal contamination as compared to other faecal coliforms, due to the fact that the faecal coliform test is non-specific and includes thermotolerant non-faecal coliforms. The enzyme, β-glucoronidase, is considered to be specific to *E. coli* and is absent in faecal thermotolerant coliforms. The presence of this enzyme confirms a presumptive positive test for *E. coli.*

This organism has been widely classified as an indicator organism for the possible contamination with faecal matter and therefore, an indication of the efficacy of the sanitation and disinfection procedures present (Montville *et al.,* 2012b, Britz *et al.,* 2012). Just the presence of *E. coli* is, however, not a direct indication of pathogenic organisms in a food or water sample, but it does increase the risk of the presence of other faecal-borne bacteria such as *Salmonella* spp. (Shtawa, 2016).

Pathogenic and non-pathogenic strains are shed together with faeces and can result in surrounding water supplies becoming contaminated, thereby, obtaining the reputation of indicating faecal contamination in water supplies (Olivier, 2015, Mahmud *et al.,* 2019). Non-pathogenic strains of *E. coli* are able to colonise as soon as a few hours after birth in the gastrointestinal tract of infants. These strains multiply within the gut and function to prevent the growth of pathogenic strains as well as producing B-vitamins for the body, therefore indicating that the impact of *E. coli* is not always negative (Forsythe, 2010, Olivier, 2015). Intestinally pathogenic strains can be divided into six main categories, namely Enteroinvasive *E. coli* (EIEC); Enteroaggregative *E. coli* (EAEC); Diffusely Adherent *E. coli* (DAEC);

Enterotoxigenic *E. coli* (ETEC); Enterohemorrhagic *E. coli* (EHEC) and Enteropathogenic *E. coli* (EPEC) with the linking factor being that these strains all cause diarrhoea (Clements *et al.,* 2012). Omar & Barnard (2010) note that these pathogenic *E. coli* strains have been identified in South African surface waters, sewage treatment facilities and other wastewater treatment facilities. Kaper *et al.* (2004) state that pathogenic strains of *E. coli* are able to inhabit areas of the body that non-pathogenic strains are incapable of, such as the urethra and small intestine due to specific adherence factors. These defining factors are most frequently fibrillae or fimbriae, but can include outer-membrane proteins and non-fimbrial proteins.

ETEC is infamously termed the cause of traveller's diarrhoea, as well as leading cause of diarrhoea in children in developing countries (Dai et al., 2008). This virotype has shown to produce two proteinaceous enterotoxins which result in intestinal secretions (Kaper *et al.,* 2004). Furthermore, Kaper *et al.* (2004) noted that there is a relationship between a higher prevalence of ETEC in developing countries and lower levels of colon cancer. The STa single-peptide toxin, produced by this strain, has been shown to inhibit the proliferation of colon cancer cells via a signalling cascade (Pitari *et al.,* 2003). Surface waters in both rural and urban areas of Bangladesh have tested positive for ETEC strains and other developing countries worldwide (Qadri *et al.,* 2005). ETEC infections result in symptoms such as diarrhoea, vomiting and abdominal pain (Percival *et al.,* 2004).

EAEC, according to Clements *et al.* (2012), is the second-most common cause of travellers' diarrhoea. This pathotype has been reported as an opportunistic enteric pathogen targeting patients with AIDS, as well as showing a high prevalence in children in developing countries (Clements *et al.,* 2012, Wanke *et al.,* 1998). EAEC produces heat stable enterotoxins, and has the ability to form biofilms in the colon or small intestine, which further increases the pathogenicity (Clements *et al.,* 2012, Jensen *et al.,* 2014). Multidrug resistance was reported in a study performed in southern India. Of the 64 EAEC strains tested, 75% were multidrug resistant (Raju & Ballal, 2009). The *E. coli* O104:H4 outbreak in Germany caused by bean sprouts was due to Shiga-toxin producing EAEC, which resulted in 54 deaths (Jensen *et al.,* 2014).

EPEC was the first pathogenic type of *E. coli* to be researched in detail (Kaper *et al.,* 2004), with a model of pathogeneses more complicated than other pathotypes. It is reported that a dose of 10^8-10^{10} organisms are required to induce an infection (Percival *et al.,* 2004). The adhesion of EPEC to epithelial cells via filaments, flagella and intimin, activates a secretion system. Odonkor & Ampofo (2013) describe this pathotype as having an array of virulence factors similar to those of *Shigella.* EPEC is able to stimulate an inflammatory response and is moderately invasive with the cause of diarrhoea being as a result of the intestinal cell ultrastructure changes due to attachment and effacement of the pathotype. Low

percentages of EPEC have been noted in the Plankenburg and Berg Rivers in the Western Cape, in a study performed by Ndlovu *et al.* (2015).

EIEC is known for closely resembling *Shigella* organisms, in that the bacteria are non-motile, unable to ferment lactose as well as in their genetic and pathogenic characteristics. This pathotype is renowned for its invasive capacity within the epithelial cells resulting in cell death via apoptosis (Levine, 1987, Schoeman *et al.*, 2013). The resulting illness is therefore, of great similarity to Shigellosis, which presents itself as high fevers and profuse diarrhoea.

E. coli O157:H7 is the most infamously known strain from the EHEC group, and is also known as Shiga-toxin producing *E. coli* (STEC), which is known for its ability to cause bloody diarrhoea (Odonkor & Ampofo, 2013). Bridle (2014) noted that 47 deaths were recorded from a verocytotoxigenic *E. coli* strain in Europe, where the source was found to be sprouts contaminated by water of poor quality. EHEC is able to cause HUS and kidney failure, both of which are fatal. Food sources as well as contaminated water sources have both shown to be transmitters of this strain of *E. coli.* The feature of concern for this pathotype is the extremely low infectious dose, where less than 100 cells are required for infection (Kaper *et al.,* 2004).

Afimbrial or fimbrial adhesions are responsible for the attachment of Diffusely Adherent *E. coli* (DAEC), which is reported as a non-toxin producing pathotype (Clements *et al.,* 2012). DAEC is responsible for causing acute diarrhoea in children below the age of five, and is not responsible for causing diarrhoea in adults (Clements *et al*., 2012, Servin, 2014). Abdominal pain, dehydration, fever and watery or bloody diarrhoea are commonly reported symptoms of this strain (Abbasi *et al.,* 2016). Servin (2014) adds that DAEC asymptomatically adds to the intestinal microbiota strains in adults and children.

A seventh pathotype has been defined, and is associated with inflammatory bowel diseases (IBD), which includes Crohn's disease and is called the Adherent-Invasive *E. coli* (AIEC) (Servin, 2014). Lee *et al.* (2019) state that AIEC can be internalised into macrophages, as well as being able to survive and replicate within the macrophage due to a host autophagy defect, where AIEC induces the release of tumour necrosis factors through the activation of the infected macrophage. Lee *et al.* (2019) continues, stating that it remains unclear whether AIEC strains are intestinal inflammation triggers or if their presence is a consequence of inflammation in patients with IBD.

Extra-intestinal pathogenic (ExPEC) *E. coli* such as uropathogenic (UPEC), neonatal meningitis associated (NEMEC) and sepsis associated (SEPEC) *E. coli* occur in the gastrointestinal tract of animals and humans but do not cause gastroenteritis or other common symptoms, but target organs such as the central nervous system and urinary tract (Olivier, 2015). Manges *et al.* (2019) state that ExPEC strains are infamously known for the ability to

acquire new and detrimental antimicrobial resistance genes. A study was performed on the evolution of drug resistance profiles of *E. coli* isolates obtained from clinical sources as well as animal meat between the years 1950 – 2002 in America (Tadesse *et al.,* 2012). A 7.2% multidrug resistance was noted in *E. coli* isolates in the years 1950 – 1959, which is shadowed by the alarming statistic that 63.6% of isolates obtained from 2000 – 2002 were resistant to multiple antimicrobials. When considering the increase in resistance per antimicrobial, the resistance of *E. coli* isolates from animal sources to chloramphenicol was reported to have an average of 0.3% increase per year. Tadesse *et al.* (2012) describes that gentamycin resistance in human isolates was only reported from the late 1990's and onwards, with 0% of isolates showing resistance in 1970 – 1979 to 28.1% of isolates showing resistance in 2000 – 2002. A 1.28% increase in resistance per year was reported for isolates against gentamycin.

2.6 Listeria species classification and pathogenesis

A major concern in the food industry is *L. monocytogenes.* This Gram-positive organism has become infamous for its high mortality rate, of between 20-30%, and for targeting individuals that are immunocompromised (i.e. pregnant women, babies and the elderly etc.) where it causes listeriosis (Farber & Peterkin, 1991, Forsythe, 2010). Within the genus *Listeria*, there are 20 recognised species, however, *L. monocytogenes* and *L. ivanovii* are of greatest concern as they are the only species with the ability to negatively impact human health (Montville *et al.,* 2012c). Ninety-five percent of the 13 *L. monocytogenes* serotypes belong to three serotypes, namely, 1/ 2a; 1/ 2b and 4b (Montville *et al.,* 2012c). *L. monocytogenes* is ubiquitous and hardy, and its ability to survive in conditions between 0°C and 42°C further increases concern as refrigeration temperatures of 4°C are not sufficient to inhibit growth (Forsythe, 2010). The infectious dose of this bacteria, albeit difficult to define, has been established as 100 cfu per gram or mL of foodstuff, however, the immunocompromised as well as the elderly, young and pregnant are said to be more susceptible where fewer cells may result in infection (Gouws & Liedemann, 2005, Allerberger & Wagner, 2010). Mateus *et al.* (2013) states that the infectious dose is strain and host susceptibility dependent. *L. monocytogenes* has the ability to spread from cell to cell, without release from the cell which is of great concern in blood-brain, placental and intestinal barriers (Dhama *et al.,* 2015). Mateus *et al.* (2013) states that in adults, the most common clinical form of listeriosis is meningitis. *L. monocytogenes* is responsible not only for systemic diseases, but can too result in localised infections. Localised listerial infections are normally identified in patients that suffer from underlying diseases and can present itself as skin infections, hepatitis, pleuritis and conjunctivitis amongst others (Doganay, 2003).

Pamer (2004), noted that *L. monocytogenes* was first isolated from a rabbit colony and dates back to 1926 in the United Kingdom. The next reporting of this disease came from South Africa, which was then named Tiger River Disease, and presented itself as necrotising hepatic infections. *L. monocytogenes* exists in the environment as a saprophyte, but is capable of transitioning to a pathogen when ingested by humans or animals (Freitag *et al.*, 2009). This indicates the adaptability of the bacteria and the ability to distinguish between environmental cues when inside or outside host cells (Freitag *et al.*, 2009). Pathogenicity of this bacteria inside a host cell is as a result of increased gene expression and bacterial replication (Freitag *et al.*, 2009). Environments where *L. monocytogenes* have been identified include animal feed, sewerage, water, soil, plants as well as processed and ready-to-eat (RTE) food products such as dairy, meat products and vegetables (Kathariou, 2002). *L. monocytogenes* is responsible for infecting the epithelial cells of the intestine, through infection via the gastrointestinal tract (Pamer, 2004). Once within the epithelial cell, the bacteria travel through the bloodstream to organs where they are introduced via hepatic and splenic macrophages (Pamer, 2004). Via a series of gene expressions and protein-activated actions, the microorganism is able to replicate within the host cell. These pathogen-containing cells are now blood-borne and are transported to various places around the body, most notably the brain where meningitis may occur or to the foetus, in the case of pregnant women, where *L. monocytogenes* can lead to septic abortions (Kathariou, 2002, Pamer, 2004, Forsythe, 2010, Montville, 2012c). Serovar 4b, which results in 37- 64% of cases, is most notable for its devastating effect on pregnant women and infants. Reduced gastric acidity in individuals over the age of 50 increases susceptibility of individuals to this disease (Forsythe, 2010). This severe disease has historically, it has been treated with ampicillin alone or in combination with gentamycin or streptomycin (Caplan *et al.,* 2014, Chen *et al.,* 2020). In recent years, studies have shown increases in resistance profiles to these antimicrobials, which has resulted in constant and consistent monitoring in the health sector (Caplan *et al.,* 2014). Caplan *et al.* (2014) reports that high levels of resistance of *L. monocytogenes* isolates from food and clinical sources to Cephalosporins are concerning as these antimicrobials are frequently used to treat infections when the cause is not known.

2.6.1 *Worldwide and South African outbreaks of listeriosis*

In Denmark and France, Schjørring *et al.* (2017) noted a cross-border outbreak as a result of smoked salmon which left seven individuals with listeriosis. It has been tallied that between 1 905 and 2 527 cases of listeriosis have been reported between 2013 and 2017 within the EU countries. The most notorious, however, is the recent outbreak in South Africa. Within the time period of January 2017 to March 2018, 978 laboratory-confirmed cases of foodborne

listeriosis were reported, the highest percentage of sufferers being from Gauteng (59%). Of the known 674 outcomes, 212 individuals died as a result of this illness in the world's largest outbreak ever recorded (WHO, 2018). A study performed by Ijabadeniyi *et al.* (2011) showed that 53% of water samples tested for *L. monocytogenes* were positive from the Olifants and Wilge Rivers and Loskop canal in South Africa. These rivers are utilised for the irrigation of crops such as cauliflower and broccoli (Ijabadeniyi *et al.,* 2011). This study further described a direct relationship between COD and *L. monocytogenes* in irrigation water, where higher COD values resulted in high *L. monocytogenes* results, however the mechanism is poorly understood.

2.7 Salmonella spp. and the presence in the food industry

Salmonella spp. falls within the *Enterobacteriaceae* family and include approximately 2 600 serotypes that are able to adapt to survive within a multitude of hosts (Eng *et al.,* 2015). These facultative anaerobic rod-shaped organisms are Gram-negative and are responsible for major burdens on both human and animal health, as well as the economic health of the food industry (Hardy, 2004). Wiley *et al.* (2014), describes *Salmonella* spp. as being mixed-acid fermenters which result in the production of lactate, acetate, succinate as well as ethanol. Theobald Smith and Dr Daniel Salmon were the first individuals to discover the genus *Salmonella* spp. in 1855 which was identified in the form of swine fever from the intestines of pigs (Eng *et al.,* 2015). Montville *et al.* (2012d) indicates that over 2 600 serovars of *Salmonella* have been identified, with *S. enterica* subspecies containing the greatest number of serovars at approximately 1 454. *S. enterica* is responsible for causing enteric fever, also referred to as Typhoid as the serotype is either Typhi or Paratyphi. There are no non-human or environmental reservoirs of this strain, and therefore contraction is primarily due to contaminated drinking water, food or poor hygienic practices (Ericsson *et al.,* 2005).

Besides *S. enterica,* non-typhoidal salmonellosis is caused by all other serotypes of *Salmonella,* which are found in abundance in the environment and animal hosts (Ericsson *et al.,* 2005). Two disease syndromes following the contamination of non-typhoidal *Salmonella* spp. are noted by Zhang *et al.* (2003), namely, enterocolitis and bacteraemia. The more notable of these two syndromes is enterocolitis, often referred to as gastroenteritis, which accounts for 99% of *Salmonella* spp. infections in warm-blooded animals as well as humans (Eng *et al.,* 2015). Within the years of 1939 to 1954, the prevalence of food poisoning caused by *Salmonella* spp. soared exponentially from 100 recorded causes to over 6 000 in 1954 in England and Wales. Although much knowledge has been gained over the past century regarding this organism, it still remains a major problem today (Hardy, 2004). Liu *et al.* (2018) suggested that 93.8 million cases of gastroenteritis are recorded annually, worldwide and

causes approximately 155 000 deaths. Antimicrobial resistance is not only a concern in the human health industry, but is dramatically affecting the food industry around the world. A study performed on antimicrobial resistance profiles of microbial isolates from bovine carcasses in Spain and Croatia found that 54.5 – 55.6% of *Salmonella* isolates were multidrug resistant. A total of 66% of *Salmonella* isolates from poultry and pork carcasses were multidrug resistant in Thailand (Kidsley *et al.,* 2018).

Salmonella spp. are able to attach and invade the epithelial intestinal cells, where numerous genes are responsible for the invasion, the genetic make-up of the cell determines the fate of developing enteric fever. Thereafter, the bacteria multiplies in endocytotic vacuoles which is unlike other genera such as *E. coli* and *Shigella* that duplicate in the cytoplasm of host cells (Montville *et al.,* 2012d). Non-virulent strains of *Salmonella* spp. are unable to invade the host cell which is crucial for pathogenesis. Normal host cell immune responses are blocked as the *Salmonella* spp. use a specific secretion system to produce effector proteins that alter the physical structure of the vacuole, resulting in cell lysosome protection prevention (Eng *et al.,* 2015).

2.7.1 Presence of Salmonella spp. *in water sources*

Salmonella spp. are not a stranger to surface waters, including dams, rivers, lakes and ponds that are all used for irrigation. Its presence is generally associated together with *E. coli* and occur on food crops that are irrigated with water of poor quality (Montville *et al.,* 2012d). A study performed by Liu *et al.* (2018), indicated that trace-back evidence of *Salmonella* outbreaks showed that 32.7% of the 53.4% of all foodborne outbreaks were as a result poor irrigation water quality. It also reported that the ability of *Salmonella* spp. to survive in aquatic environments can be attributed to a number of mechanisms, which includes the ability of the bacteria to enter a state of being viable but non-culturable (Liu *et al.,* 2018). The resulting effect of the three syndromes caused by *Salmonella* spp. can be observed in Table 1.

2.7.2 Antibiotic resistance of Salmonella *spp.*

Antibiotic resistance of *Salmonella* spp. has been noted since the early 1960s, where an individual developed resistance to chloramphenicol, and has become a cause for major concern in recent years (Eng *et al.,* 2015). The genes that determine resistance to antibiotics are often on the same plasmids as those that determine *Salmonella* spp. virulence (Montville *et al.,* 2012d). Antibiotics such as ampicillin, chloramphenicol and cephalosporin's are routinely given for individuals with *Salmonella*-caused syndromes, and in areas where resistances have been identified, fluoroquinolones are provided instead (Acheson & Hohmann, 2001, Dyson *et al.,* 2019). Individuals in certain areas of South Asia have been

identified as being non-susceptible to fluoroquinolones but this drug has proven to be susceptible in most countries of the world (Ryan & Andrews, 2019). It was identified in 2018 that 75% of strains of *Salmonella* spp. from African countries can be regarded as multidrug resistant. This study continued to note that extreme drug-resistance (XDR) strains of *S. typhi* were recorded in over 5 000 individuals worldwide (Ryan & Andrews, 2019).

Table 2 can be used as a general guide of the infectious dose, symptoms and symptoms for specific pathogens as well as the most likely sources of contamination as determined by previous outbreaks.

Table 1 Symptoms and descriptions of diseases caused by infection with *Salmonella* spp.

Syndrome	Description	Symptoms	Method of identification	Prevention & cure	Reference
Enteric fever *S. enterica* - serovar Typhi or Paratyphi	Typhoid and paratyphoid clinical symptoms are indistinguishable, incubation period of 7- 28 days	Low-high grade fever, abdominal pain, diarrhoea, headaches and possibility hepatomegaly, splenomegaly and myalgia.	Blood samples or urine testing in early stages or stools from onset of symptoms	Typhoid vaccinations, antibiotics, avoid unpasteurised dairy products and undercooked fish and meat	Montville *et al.,* (2012d), Zhang *et al.,* (2003), Eng *et al.,* (2015), Ryan & Andrews (2019).
Enterocolitis *S. typhimurium, S. newport, S. enteritidis*	Inflammation of the digestive tract, lasts 3- 4 days on average	Abdominal pain, vomiting, diarrhoea and nausea	Blood samples or urine testing in early stages or stools from onset of symptoms	Cook meats thoroughly, ensure general hygiene, boil water before drinking in high risk areas antibiotics, fluid and electrolyte replacement	Srikanth & Cherayil (2007), Montville *et al.,* (2012d)
Bacteraemia *S. dublin, S. heidelberg, S. choleraesuis*	Bacteria enter bloodstream, almost all serotypes can cause this, targets immunocompromised, complication of gastroenteritis	High fever, severe conditions can lead to septic shock, abscess formation, pneumonia	Blood sampling, urine and stool tests	Intravenous antibiotics, good hygiene practise, ensure eggs and meat products are sufficiently cooked	Eng *et al.,* (2015), Mileva *et al.,* (2016), Cianflone, (2009).

Table 2 Microorganisms under investigation in this study and their effect on human health as well as sources of contamination

Microorganism	Infectious dose	Incubation period	Symptoms	Source of contamination	Reference
E. coli (O157:H7)	10 – 100 CFU	3 - 4 days	Watery diarrhoea, low grade fever, stomach cramps	Raw meat (beef), dairy products, fresh produce, faecal matter and water sources	Baylis *et al.* (2011), Rahal *et al.,* (2012)
L.monocytogenes (listeriosis)	10 – 10 million CFU in healthy individuals 0.1 – 10 million CFU in high risk individuals	1 - 4 weeks Febrile gastroenteritis from *L. monocytogenes* *18 – 20 hours*	Fever, vomiting, diarrhoea, weakness, confusion	RTE foods, dairy products, poultry, vegetables and water sources	Schweon, (2015)
Salmonella spp. (enteric fever)	20 – 10^6 CFU depending on health of individual	12 - 36 hours	Headache, abdominal pain, diarrhoea or constipation, fever	Eggs, poultry, pork, beef, dairy, fruits and vegetables and faecal matter	Forsythe, (2010), Eng *et al.,* (2015), Andino & Hanning (2015)

2.8 Irrigation, domestic and drinking water guidelines in South Africa

Physical, biological, chemical and aesthetic attributes are used to describe the quality of water used in different environments and, therefore, the fitness for use (DWAF, 1996a). The microbial quality of irrigation and drinking water within South Africa has become a cause for major concern, studies show (Huisamen, 2012, Britz, 2012, Britz, 2013, Omar & Barnard, 2010). *E. coli* counts of between 230 and 7 x 10^6 CFU. 100 mL^{-1} were identified in the Plankenburg and Eerste Rivers in the Western Cape in a study performed by Huisamen (2012). The crops irrigated with water from these rivers were investigated and noted that various microorganisms such as *L. monocytogenes, Staphylococcus aureus, E. coli* and *Klebsiella* spp. were present. Therefore, the need to meet requirements and guidelines for water safety and quality is ever increasing. In 1996, DWAF developed a set of guidelines for water safety in use in specific areas. These guidelines include sections for water used recreationally, agriculturally, domestically as well as industrially used water. Water guidelines differ between countries, and can be attributed to the economic status of the country, as well as the perceived risks associated with certain organisms, based on the history of contamination and outbreaks in each country (Steele & Odumeru, 2004). High treatment cost, lack of facilities, equipment and knowledge in some developing countries result in water of low quality, and ultimately lower standards as compared to developed countries. In Tables 3 and 4, the South African microbiological and physico-chemical guidelines for water used for irrigation purposes, domestic use and fresh produce can be found, and need to be met in order to be deemed acceptable for use.

Table 3 Guidelines associated with the microbiological aspects of water in South Africa obtained from DWAF Vol 1 & 4 (1996a & c) & Department of Health (2002)

	Microbial load		
Microorganism	Agricultural irrigation	Domestic use	Fresh Produce
E. coli	< 1000 cfu.100mL^{-1}	< 10 cfu.100mL^{-1}	0 cfu.g^{-1}
L. monocytogenes	NS	NS	0 cfu.g^{-1}
Salmonella spp.	NS	NS	0.25 g^{-1}
Protozoan pathogens	< 1 cyst	< 1 cyst	0

NS – not specified

Knowledge gaps exist in South Africa with regard to the prevalence of *Salmonella* spp. and *L. monocytogenes* in irrigation water. This can result in underreporting of these organisms as there is no legislative pressure to test for them. The inclusion of the food pathogens, *Salmonella* spp. and *L. monocytogenes*, in the current study is as a result of the risk that is

associated with these organisms in South African water systems. *L. monocytogenes* has been identified as an organism found naturally in the environment (Olaimat & Holley, 2012). *Salmonella* spp. and *E. coli* are the primary organisms found in faecal matter of cattle and sheep and therefore, contamination of water sources is incredibly likely (Olaimat & Holley, 2012). *E. coli* is an organism that has been widely used as an indicator organism in water and food sources for the presence of faecal contamination. A report by Sigge *et al.* (2016) describes the importance of disinfection of food pathogens in addition to *E. coli,* such as *L. monocytogenes, Salmonella* spp. and protozoan pathogens need to be considered in the food industry to ensure a reduction in foodborne outbreaks.

Total dissolved solids (TDS) and electrical conductivity (EC) are interconnected attributes, with a directly proportional relationship. Electrical conductivity of a water sample describes the ability of water to conduct an electric current, and this relies on the presence certain substances such as sulphates, nitrates, sodium and calcium ions which carry an electric charge. Crops are, however, sensitive to soil salinity and therefore, a control measure needs to be maintained with regard to the salt content which will accumulate in the soil. The conversion between TDS ($mg.L^{-1}$) and EC ($mS.m^{-1}$) for most waters can be obtained by multiplying the EC value by 6.5. (DWAF, 1996a).

Table 4 Guidelines associated with physio-chemical aspects of water used for agricultural irrigation and domestic purposes in South Africa, obtained from DWAF (1996a) & DWAF (1996c)

Quality	Agricultural Irrigation	Domestic use & drinking water
pH	6.5 – 8.4	6.0 – 9.0
TDS ($mg.L^{-1}$)	260	0 – 450
EC ($mS.m^{-1}$)	40	0 – 70
TSS ($mg.L^{-1}$)	50	50
Turbidity (NTU)	NS	0 – 1
Alkalinity (mg $CaCO_3.L^{-1}$)	<120	50 – 100

NS – not specified

The chemical oxygen demand (COD) is defined as the oxygen equivalent of the total organic matter within a sample that may show susceptibility to oxidation as a result of a strong chemical oxidant (DWAF, 1996a). This aspect is important to consider in water samples as CODs higher than the stipulated value can cause damage to machinery as well as interfere with treatment efficacy (DWAF, 1996a). This value should be interpreted with the turbidity of the sample. Currently, there is no stipulation within the Water Quality Guidelines (WQG)

(DWAF, 1996a) in South Africa regarding the COD of a water sample for either agricultural irrigation water or domestically-used water. Therefore, the WQG for Industrial Use (Vol 3) were consulted instead (DWAF, 1996b). This document states that acceptable limits for COD in irrigation water is < 75 mg $O^2.L^{-1}$ as irrigation water falls within category 4 Utility Water of Industrial Processes, which allows for the discharge of an effluent. Drinking water COD limits were determined to be 0 – 30 mg $O^2.L^{-1}$ from Category 3 Product Water in the WQG for Industrial Use (DWAF, 1996b).

The turbidity of a water sample is as a result of the suspended solids in the water. These measurements are dependent on the particle size distribution of the matter that is suspended in the solution. Settling within the water, is as a result of the coagulation of matter due to the neutralisation of electrical charges by salts of Al (III) and Fe (III). Turbidity is of great importance to individuals in the agricultural industry as high turbidity values can result in damage to irrigation equipment such as sprinklers as well as negatively affecting the appearance of crops (DWAF, 1996a). Turbidity is measured in nephelometric turbidity units (NTU), with water of one NTU being transparent and 1 000 NTU being muddy and opaque.

Water hardness or alkalinity values can be used to determine the likelihood of corrosion to pipes as well as the ability to form scale build-up. Various indices can be utilised to determine the value at which water is saturated with calcium carbonate (amongst other salts), i.e. the Langelier index, the Aggressiveness index or the Ryznar index (DWAF, 1996a). In drinking water regulations, however, the total hardness is expressed as the mg $CaCO_3.L^{-1}$. The guidelines for agricultural irrigation water express total hardness by using either of the three aforementioned indices (DWAF, 1996a).

The WQG (DWAF, 1996a) provide a general guide of approved and allowed water quality constituents, and with which the fitness for use can be decided based on the constituents falling within these suggested ranges. Any constituent that is not contained within the suggested limits or ranges, deems the water unfit for use in the specific environment. A more holistic approach is now under investigation, which is site-specific, risk-based and takes a variety of considerations into account for deciding the fitness for use of water samples. This is in the form of a Decision Support System (DSS) for the testing and use of irrigation water, that will ultimately be used for domestic and recreational water use as well as irrigation water (Du Plessis *et al.,* 2017). These new guidelines have the aim of providing both general and site-specific recommendations of the quality of water used for agricultural irrigation purposes and uses tiers as a form of hierarchy.

Tier 1 is the first port of call for considering water quality, is equivalent of the WQG as described by DWAF (1996a), and provides a water quality assessment, albeit conservative,

that indicates areas of concern in the sample. If there is a potential problem indicated by Tier 1 testing, Tier 2 testing should be used to determine fitness for use in a site-specific and in-depth manner by using the DSS. This is a sophisticated model that incorporates a variety of factors such as soil-water balance and chemistry to determine water quality in different climatic and water management environments. Thereafter, significant expertise is required for Tier 3 testing, which is of a specialised nature. Tier 3 investigations do not fall within the DSS, however, some guidance for testing is provided (Du Plessis *et al.,* 2017). In terms of microbial contamination of water samples, the revised guidelines describes the risk of a specific pathogen by indicating the amount of infections per 1 000 individuals per annum for Tier 1 investigations, with lettuce assumed to be the most sensitive crop for contamination. Tier 2 uses a more specific approach through the use of crop specific parameters. This is opposed to the Irrigation Water Guidelines which state the amount of colonies that would cause an infection after consumption (DWAF, 1996). It is, therefore, acknowledged that there are newer and updated standards provided by DWAF. However, due to the fact that these new guidelines do not include specific limits for the parameters under investigation, for the current study, the older standards (DWAF, 1996a, DWAF, 1996b, DWAF, 1996c) were used so as to have values to compare to as a standard reference.

2.9 Treatment methods for irrigation water

The NHMRC (2004) states that physical, chemical or photochemical methods can be employed for the disinfection of water samples, which is aimed at lowering, deactivating or removing organisms that may negatively impact the health of individuals. This is, therefore, done to meet the guidelines provided by the country, to reduce the risk of widespread outbreaks and impact the economic status of the country due to illness, death, inability to work and huge losses due to recalls of fresh produce contaminated by irrigation water. The WHO (2010) stated that the use of contaminated water in developing countries for agricultural irrigation purposes is a common occurrence and needs to be addressed to reduce health risks.

2.9.1 Physical methods utilised for water disinfection

Physical methods are referred to as filtration methods that are utilised for the separation of solid particles and other particulates from liquids and has been classified as the oldest method of disinfection for water (Kesari *et al.,* 2011). Slow sand filtration and ultrafiltration are the most commonly used examples of physical disinfection methods for irrigation water. The efficacy of physical treatment methods is limited by the physical size of the pores in the filter medium, however, a major advantage being that these filtration techniques impart no negative flavour on the water being treated (Okpara *et al.,* 2011).

Slow sand filtration as a physical treatment method

Slow sand filtration (SSF) has been described as an effective measure of water disinfection in developing countries due its simplicity and affordability (Keraita *et al.,* 2008). WHO (2010) state that SSF methods are generally used as filtration methods for water that is irrigated with a drip irrigation system. This is achieved by passing water through a bed of sand or pumice, to remove both physical and biological pathogens (Zheng & Dunets, 2014). The extent to which the water is filtered is dependent on the size of the sand particles, with smaller sizes increasing the filtration efficacy. This, however, also directly increases the need for frequent cleaning by backwashing (Haman & Zazueta, 1994). Haman & Zazueta (1994) states that the efficacy of filtration is inversely proportional to the filtration flow rate. The system consists of a water layer, schmutzdecke layer, filter bed and then a layer of gravel of specific depths which each play a specific role. Microorganism levels are reduced through the action of the schmutzdecke layer, which acts as an active biofilm (Zheng & Dunets, 2014). Olivier (2015) and Hendricks (2006) state that the microorganisms within the water will be outcompeted for resources by the active biofilm which is effective at reducing levels of bacteria, cysts, viruses and algae by between 2- 4 log reductions. Zheng and Dunets (2014) recommend the use of a pre-filtration step before slow sand filtration due to high particle levels continuously blocking the filter pores.

Advantages and Disadvantages of the SSF method

Advantages of this method include:

- Simple technology
- No harmful chemicals
- Low operating costs

Disadvantages of this method:

- Decreased performance due to variability in filter porosity
- High installation costs
- Frequent clogging, resulting in constant maintenance requirements

(Zheng & Dunets, 2014)

Membrane filtration as a method of physical and biological disinfection

Membrane filtration includes microfiltration (MF), ultrafiltration (UF) and nanofiltration (NF) and has been used for decades as a means of treating water for various uses by using membranes with pores of specified sizes (Zularisam *et al.,* 2006). The membrane, in this case, is a synthetic and polymeric barrier that is selective for certain constituents within the sample, which is dependent on the application (Zeman & Zydney, 2017). According to Leiknes (2009),

the use of MF has shown efficacy in the removal of pathogenic microorganisms and some viruses whereas NF is effective for the removal of all viruses from water. Most applicable to the requirement at hand is the use of UF which is able to treat water to a point where it falls in line with water quality guidelines and is the most common type of membrane used for this application. Ultrafiltration is effective at removing inorganic particles, microorganisms as well as dissolved organic matter (Zularisam *et al.,* 2006).

Important factors to consider with the use of membrane filters are the thickness and surface area of the membrane, the total inorganic matter of the water sample, as well as membrane fouling (Pellegrino, 2011). Fouling is described by Pellegrino (2011) as a decline in flux as a result of accumulation or adsorption of matter on the surface and within the pores of the membrane as a result of solutes or precipitates. This is the largest obstacle to overcome when using membrane filtration methods, as reduction in efficacy increases costs as productivity is reduced (Zularisam *et al.,* 2006).

The advantages of using this method of disinfection include the elimination of possibly harmful chemicals and therefore, prevents the development of harmful by-products, and an effective pre-treatment procedure for water samples (Jacangelo & Noack, 2005). Limitations of this process include reduced flux due to fouling, irregular pore sizes preventing filtration uniformity and high capital costs (Momba *et al.,* 2008).

Glass bead media filters as a method of water purification

Glass bead filters have become an ever-increasing interest in the field of water treatment as an alternative to slow sand filtration techniques (Klaus, 2015). This is due to the fact that slow sand filtration methods have shown several limitations such as decreased flow rates, high levels of particle accumulation causing caking and extensive backwashing requirements to maintain effective flow rates, which can also lead to loss of filter media. Most importantly, sand filtration methods prove to be difficult to prevent bacterial growth within the media due to sand being an excellent substrate for growth (Al-Aibi *et al.,* 2018). Investigations into activated glass bead media filters showed exciting results; as solids within the water are held back within the glass beads by weak electric forces such as London and Van Der Vaal's Forces, backwashing would release all particles with ease compared to the sticking of particles within sand filters (Al-Aibi *et al*., 2018). Furthermore, activated glass bead filters are able to improve water clarity by reductions in NTU of approximately 25%, water usage for backwashing can be reduced by up to 23% and lastly, up to 20% less media is required (by weight) as compared to sand filtration (Al-Aibi *et al.,* 2018). Klaus (2015) describes the use of this technique as having enhanced performance and lifetime cycles as compared to sand filtration methods.

In conclusion, there are many advantages as well as limitations to using physical disinfection for water. Physical methods are generally seen as a pre-treatment for water and wastewater treatment. It is, therefore, advisable to use them in conjunction with another method of treatment for optimal results.

2.9.2 Chemical methods utilised for the disinfection of water samples on a large scale

The requirements for an effective disinfectant are described by Tchobanoglous *et al.* (2003) as guaranteeing the maximum disinfection of pathogenic organisms, without the generation of toxic by-products. These compounds should be inexpensive, with simple application resulting in water of a consistent and specific quality (Tchobanoglous *et al.,* 2003). Linley *et al.* (2012) note that the demand for non-toxic, environmentally friendly and potent chemicals has never been higher, not limited to water treatment methods but also for medical, food and industrial applications. Chemical treatment methods are generally applied to water samples alone or in combination with physical treatment methods such as filtration, Ultraviolet treatment or advanced oxidation processes (AOPs). According to Comninellis *et al.* (2008), the term AOPs can be defined as the oxidation of an aqueous phase, based on highly reactive species, resulting in the destruction of a pollutant. Chemical treatment methods are often accompanied by the formation of carcinogenic by-products and therefore, the need to investigate and utilise chemicals that reduce the formation of disinfection by-products (DBPs) in the environment is ever-increasing (Jyoti & Pandit, 2004). Peracetic acid, chlorine, ozone and hydrogen peroxide are the four most notable chemicals that are employed for water disinfection purposes (Jyoti & Pandit, 2004).

Chlorine – introduction & mode of action

Chlorine has been an integral component of the disinfection and purification industry for two centuries, and was the gold standard process for treating wastewaters to improve public health. Within the last 60 years, however, the environmental impact of this chemical began to cause concern worldwide (Whitby & Scheible, 2004). By the 1980s, great emphasis was placed on legislation and regulations regarding the use of chlorine due to hazards, by-product formation and residuals in the treated water samples (Whitby & Scheible, 2004). Hrušková *et al.* (2018) states chlorine in the form of gaseous chlorine, sodium or calcium hypochlorite, chloramines and chlorine dioxide have been utilised for disinfection purposes, each with their own set of advantages and limitations.

The bactericidal effect of chlorine can be attributed to the disruption of protein synthesis, alteration of cytoplasmic membranes and decreases in nutrient transport (Virto *et al.,* 2005). Okpara *et al.* (2011) continues to explain that chlorine, in sufficient doses, is able to disinfect water samples of microorganisms within 30 minutes. Chlorine is able to oxidise organic

compounds within water samples, and reacts with water molecules to form hypochlorous acid (HOCl), and then OCl^{-} by dissociation which are both responsible for the antimicrobial properties of chlorine. Trials by Lavonen *et al.* (2013) showed that a decreased water pH increases the bactericidal efficacy of the chlorine, which is as a result of the formation of hypochlorous acid, which enhances the ability of hypochlorous acid to diffuse through cell membranes.

Richardson *et al.* (2000) denotes that the biggest drawback of chlorine is the production of DBPs. Over 600 DBPs have been reported in literature, with four most notable being chloroform, bromodichloromethane, bromoform, 3-chloro-4-(dichloromethyl)-4-oxobutenoic acid and trihalomethanes. These DBPs have proven to be carcinogenic for laboratory animals (Richardson *et al.,* 2007). Benson *et al.* (2017) explains that not only are some DBPs carcinogenic, but they have shown to be cytotoxic, genotoxic, mutagenic and teratogenic. The formation of these DBPs is dependent on the temperature, pH, water source, type of disinfectant utilised and exposure time (Benson *et al.,* 2017). One of the most severe drawbacks of the formation of the DBP's is the development of cross-resistant as well as antimicrobial reisistant bacteria (Li & Gu, 2019). This occurs via genetic mutations or horizontal gene transfer.

Momba *et al.* (2008) provides an indication of the dosage requirements for effective treatment, which is 2-3 $mg.L^{-1}$ which is able to cause a 3-log reduction in the microbial load. The use of chlorine for disinfection proves to be advantageous for the reduction in foul smells and tastes within water samples, which is achieved through oxidation reactions (Momba *et al.,* 2008), however increased chlorine concentrations can result in undesirably tasting water. Dechlorination is utilised for the removal of residual chlorine causing undesirable tastes, however, increases the cost of chlorination by up to 50% (Solomon *et al.,* 1998). Chlorine treatment is cost effective, with low installation costs further increasing the attractiveness of the compound.

The limitations of this treatment method include; DBPs, negative environmental impact, highly corrosive compounds, microbial resistances to the compound causing decreased disinfection efficacy, slow treatment time and the requirement for holding tanks increasing expenses (Solomon *et al.,*1998).

Peracetic acid as an effective disinfectant

In an attempt to reduce the production of harmful DBPs, research was conducted to identify an alternative to chlorine for the disinfection of water samples. Peracetic acid (PAA) therefore, showed potential as a disinfectant that has little to no potential for DBP formation (Zhang *et al.,* 2019). Other than being an effective disinfectant for water treatment, PAA can be used for

disinfection in ion exchangers, cooling towers and membrane hollow fibres. Other applications include that of the medical industry, beverage and food processing industries (Kitis, 2004, Zanetti *et al.,* 2007). Kitis (2004) states that the oxidation potential of PAA is greater than that of chlorine as well as considered to have greater disinfectant power than hydrogen peroxide due to its efficacy of disinfecting a wide spectrum of microorganisms at low concentrations. This low pH (<2), pungent compound is completely soluble in water and is produced from the following reaction:

$$CH_3CO_2H + H_2O_2 \rightarrow CH_3CO_3H + H_2O$$

(Kitis, 2004).

This bactericidal, fungicidal and sporicidal compound quickly became an attractive disinfectant, however, suspended solids decrease the efficacy of this compound by shielding the bacteria from the disinfectant as well as by consuming the PAA, thereby, reducing the concentration available for microbial disinfection. It is therefore, important to ensure that there is sufficient disinfectant to achieve the targeted application, whilst avoiding high residuals (Kitis, 2004, Henao *et al.,* 2018). The main factor to consider is the dosage ($mg.L.min^{-1}$) which determines the overall disinfection efficacy. A study performed by Chhetri *et al.* (2014) regarding the efficacy of disinfectants in wastewater treatment noted that with the use of PAA, *E. coli* levels were reduced to undetectable levels, however, Enterococci disinfection efficacy was weaker, indicating that these organisms are more resistant to the chemical. However, dosages of approximately 5 - 10 $mg.L^{-1}$ were sufficient for 3 – 5 log reductions in coliforms, faecal coliforms and *E. coli.*

The poorly understood mode of action of PAA is noted by Kitis (2004) as being based on the release of an active oxygen molecule which affects the sulfhydryl and sulphide bonds, located within the microorganism's enzymes, and proteins by oxidising them. This, then disrupts the chemiosmotic function of the lipoprotein cytoplasmic membranes or causes rupturing of the cell walls. Decomposition by-products of PAA include acetic acid, hydrogen peroxide, oxygen and water and has shown to produce no mutagenic by-products following reactions with organic material. De Luca *et al.* (2008) states that the use of PAA is primarily in wastewater disinfection and less so for the treatment of agricultural irrigation water, and that this could be attributed to the fact that it's a relatively new compound in the industry, only gaining momentum in the past 20 years.

Hydrogen peroxide and its application as a disinfectant

Hydrogen peroxide has gained momentum as a disinfectant since its discovery as a chemical in 1818, but only later regarded as an effective antimicrobial and antiseptic compound (Litter *et al.,* 2014). Drogui *et al.* (2001) describe hydrogen peroxide as a compound of high redox

potential, with disinfection properties being a result of direct molecular action as well as free radicals that are formed from catalytic reactions. The free radicals, in the presence of oxygen, cause numerous oxidative reactions to cascade which results in the destruction and decomposition of enzymes and organic matter within the water samples. This, ultimately leads to the rupturing of cell walls, which results in cell death (Litter *et al.,* 2014). Hydrogen peroxide is produced in one of two ways, either chemically or via electrolysis. The efficiency of this disinfectant is dependent on a number of factors that include; the cell structure, dosages, treatment time as well as environmental conditions (Litter *et al.,* 2014). Giddey (2015) and Kumar & Pandit (2013) highlight several disadvantages of the use of this chemical, which include the danger if in contact with skin, and the flammable and explosive nature which is due to its strong oxidant ability. Furthermore, high levels of organic matter in the sample may limit disinfection efficacy.

A study performed by Drogui *et al.* (2001) on the dosage requirements for hydrogen peroxide in municipal sewerage effluents noted that a dosage of 15 $mg.L^{-1}$ is required to remove organic compounds and decrease turbidity. Ronen *et al.* (2010) noted that a concentration of 125 $mg.L^{-1}$, with a contact time of 35 min, is required for a 99% reduction of faecal coliforms. Van Haute *et al.* (2015) and Giddey (2015) describe that post-harvest washing of fruits and vegetables with water containing specific doses of hydrogen peroxide showed potential due to the inability of the formation of DBPs, as well as no long term residuals. Hydrogen peroxide used for disinfection in the drinking water industry has proved to be relatively unsuccessful due to the high concentrations required, and therefore, high costs to ensure safety for consumption. This chemical is primarily used for the production of potable, but not drinkable water, according to Kumar & Pandit (2013). Even then, the use of this chemical as the primary disinfectant is limited, which has resulted in the use of combination treatments to produce water with specific requirements for safety purposes. Research shows that the combination of hydrogen peroxide and ozone, UV light or metal ions can increase the disinfectant capacity of this chemical (Giddey, 2015, Kumar & Pandit, 2013, Van Haute *et al.*, 2015). Van Haute *et al.* (2015) noted that the inclusion of a commercial metal ion formulation, which contains Cu^{2+}, Zn^{2+} and Ag^{2+} provided an improved stability of hydrogen peroxide in water samples and showed potential for use in off-line processes. Giddey (2015) noted that when hydrogen peroxide was used in combination with Cu^{2+}, a 3.9 log reduction in faecal coliforms occurred. While the use of hydrogen peroxide alone, at the same concentration, allowed for a 0.9 log reduction in faecal coliforms in the same water sample. This study also concluded that the use of Ag^{2+} with hydrogen peroxide was not as effective as Cu^{2+} with hydrogen peroxide. A study performed by Raffellini *et al.* (2011) noted that *E. coli* cells that are attached to other components such as suspended organic material within

the water sample were more resistant to H_2O_2 treatments, which increases concerns for treatment efficacy and dosage requirements to overcome this downfall. Giddey (2015) noted that the application of UV radiation in combination with hydrogen peroxide increased the log reductions as compared to using UV radiation alone. The application of this chemical in combination with ozone was described by Kumar & Pandit (2013) as an advanced oxidation process.

The efficacy of ozone as a disinfectant

Ozone's application in the water industry as a disinfectant began in 1886 in France, which is considered to be the cradle of this chemical. Within 14 years of initial research, over 700 water treatment plants were under operation in France. Most notably, in 1906, a water treatment plant was able to treat over 22 000 m^3 of water per day after slow sand filtration (Loeb *et al.,* 2012). According to Loeb *et al.* (2012), the use of ozone remains the first choice treatment for drinking water in France. Ozone treatment in the U.S. has two notable applications for water treatment, namely; the removal of harmful by-products in the primary disinfection step as well as microorganism inactivation in secondary disinfection (Rojas-Valencia, 2011). Ozone (O_3) is produced when an oxygen molecule (O_2) is treated by an energy source (voltages up to 20 kV) causing its dissociation and collision with another oxygen molecule to form the unstable gas (O_3) (Boner & Weston, 1999). Once the ozone dissolves in water, hydroxyl and hydrogen-peroxy radicals form which both have a greater oxidizing capacity; further increasing the disinfection efficacy (Boner & Weston, 1999).

Ozone is an effective treatment for viruses in water as it is able to oxidise envelope proteins and cause cell structure modifications, a process that is relatively simple due to the uncomplicated structure of the virus envelope. Fungi can be eliminated by ozone in water samples, via an irreversibly damaged cell structure. A bactericidal and bacteriostatic effect is noted by ozone, at low concentrations and treatment times (Rojas-Valencia, 2011). This is as a result of the oxidation of the protoplasm and membrane, followed by an alteration of the buffering mechanism of the cell leading to cell death. Spores develop in unfavourable conditions and are able to withstand environmental stress with increased resistances as compared to fungi and bacteria. Increased dosages of ozone are thus required to overcome this resistance. Protozoan pathogens are major concerns within water samples, and can be effectively treated using ozone, most notably *Giardia lamblia* and *Cryptosporidium* spp. The latter being almost ten times more resistant to treatment by ozone than the former (Rojas-Valencia, 2011).

Disinfection with ozone has been described as more effective than chlorine with regard to the destruction of bacteria and viruses, as well as a shorter contact time required. The

development of harmful DBPs is prevented and no residuals remain in the water. The use of ozone treatment in water increases the dissolved oxygen concentration, which eliminates the need for aeration downstream (Boner & Weston, 1999). A study performed by Raub *et al.* (2007) noted that not only does ozone treatment have a positive effect on producing irrigation water of an acceptable quality but increases the soil quality when crops are watered with ozone-treated water. Raub *et al.* (2007) indicated that an increase in water penetration, crop vigour and less free-standing water was achieved through the use of ozone. Limitations of this method include that disinfection efficacy can be impaired with high amounts of particulates, biological oxygen demand (BOD) and COD in the water sample, shielding the bacteria from direct treatment. This is an expensive treatment method that is not user-friendly, requiring complicated equipment, energy input and storage equipment due to the high corrosivity of the chemical (Boner & Weston, 1999).

2.9.3 Photochemical methods of water disinfection

Photochemistry involves chemical reactions occurring between atoms or molecules that have been excited by the absorption of electromagnetic radiation within the wavelengths of 170 nm and 700 nm (Pfoertner & Oppenlander, 2012). Photochemical processes are versatile and efficient methods of disinfection, which are able to effectively inactivate a wide variety of pathogenic and indicator organisms within food and water sources (McEvoy & Zhang, 2010). This treatment method has gained exponential momentum as knowledge around the effect of harmful chemicals that form DBPs were identified. Alternatives became imperative to reduce the harmful effect on the environment, as well as animal and human health and therefore, moving towards a sustainable future. The push towards a “green” future in technology will further encourage the use of photochemical processes (Galvéz & Rodrígues, 2010). Advanced oxidation processes (AOPs) involve the utilisation of Ultraviolet technology in combination with hydrogen peroxide, ozone or other chemicals and medium-pressure mercury lamps (Pfoertner & Oppenländer, 2012). The first evidence of photochemical AOPs was introduced by Glaze in 1987 where it was reported that hydroxyl radicals could be generated using photons of energy provided by a light source, in a sufficient amount to affect the purification of water samples (O’ Shea & Dionysiou, 2012). The use of AOPs has successfully shown to decrease the production of DBPs in water samples and therefore, provides a greater advantage over the use of chemicals alone (Otturan & Aaron, 2014). The main advantage of this method include that the organic components are oxidised to carbon dioxide, which is a non-toxic compound. Electron beam technology provides a method of sanitisation that is not only effective for water treatment, but an extensive list of applications. Kurilova *et al.* (2015) state that electron beams achieve the bactericidal effect through the use of ionising radiation to directly (physically) and indirectly (chemically) cause microorganism destruction.

Photochemical treatment methods, are thus, an effective measure of ensuring water quality and safety.

Advanced oxidation processes in water disinfection

Initially only hydroxyl radicals, and later the introduction of sulfate radicals were discovered as effective molecules for water decontamination. This disinfection method was first introduced in 1980 to produce potable water from effluent, through the use of potent oxidising agents (Deng & Zhao, 2015). This process can be described using the term AOPs where the primary goal is the destruction of organic and inorganic compounds within water samples using highly reactive and non-selective radicals (Sherchan *et al.,* 2014). Deng & Zhao (2015) explain that the use of AOPs for the inactivation and destruction of pathogenic and indicator organisms is limited due to the short half-life of the radicals, therefore indicating that this method of inactivation cannot be effectively employed as the primary method of disinfection for water samples and should be used in combination with other methods to ensure consistent microbial disinfection. Hydroxyl radicals are responsible for targeting the cell membranes, proteins and lipids of the microbial cell, which results in damage to genetic material ultimately leading to the inability of cells to perform normal transcription, translation and replication (Linley *et al.,* 2012). Equation 1 – 3 can be used to describe the process of hydroxyl radical formation as indicated by Oturan & Aaron (2014):

$$H_2O_2 + UV \rightarrow 2\ \bullet OH \quad (1)$$

$$\bullet OH + H_2O_2 \rightarrow H_2O_2 + HO_2\bullet \quad (2)$$

$$HO_2\bullet + H_2O_2 \rightarrow \bullet OH + H_2O + O_2 \quad (3)$$

Chemical, photochemical, sonochemical and electrochemical AOPs have been described by Oturan & Aaron (2014). Chemical AOPs involves the utilisation of Fenton's regent, which is a combination of H_2O_2 and Fe^{2+} resulting in the decomposition of various organic water contaminants. Advantages of this method include no energy input requirements, relatively inexpensive chemicals as well as simple operating procedures. High costs due to transport and storage of chemicals, accumulation of iron sludge and over-mineralisation of the water from Fe(III)-carboxylic acid complexes that cannot be destroyed by the hydroxyl radicals, are but a few of the limitations of this treatment method (Oturan & Aaron, 2014).

Braun & Oliveros (1997) describe photochemical AOPs as using Ultraviolet light combined with hydrogen peroxide or other chemicals such as ozone (O_3) or PAA, to reduce dissolved and dispersed organic compounds with great efficiency. Ultraviolet radiation between 200 – 300 nm is required to initiate the production of the radicals. The use of either low- or medium-pressure UV radiation will be sufficient for this application (Oturan & Aaron,

2014). The use of photochemical AOPs has also successfully shown to decrease the production of DPBs in water samples (Oturan & Aaron, 2014). Van Rooyen (2018) describes that not only are photochemical AOPs effective for reducing organic matter, but also for the reduction of pharmaceutical, pesticides and negatively tasting compounds in water samples. Sherchan *et al.* (2014) noted that a complete microbial inactivation was experienced in the treatment of a water sample where a combination of UV and H_2O_2 was utilised. Another study performed by Sanches *et al.* (2010) described the three modes of action for this treatment method, the first being that the germicidal effect of the process is as a result of direct photolysis due to the UV radiation. The second being the oxidative damage that is as a result of the chemical used (be it O_3, H_2O_2 or PAA). Lastly, the oxidation by photocatalysis from the hydroxyl radical formation. Oturan & Aaron (2014) state that the use of photochemical AOPs for water treatment is more effective as well as of greater economic value than chemical AOPs.

Vulhunen & Sillanpää (2010) as well as Oturan & Aaron (2014) describe the use of ultrasound (US) as a sonochemical process of enhancing the efficiency of conventional AOPs in a direct or indirect mechanism. The direct method, also called sonication, utilises the formation of cavitation bubbles that form and collapse at extremely high temperatures and pressures which allows for the formation of highly reactive radicals, leading to the oxidation or reduction of organic and inorganic molecules. The indirect mechanism of this process involves the combination of water and dioxygen molecules, at high US frequencies, that undergo a process of homolytic fragmentation to produce hydroxyl-, hydrogen peroxide- and oxygen-radicals (Oturan & Aaron, 2014). Huang *et al.* (2011) describe that in the United States, little effort is made to reuse secondary effluents to create potable water to be used in agricultural irrigation systems. In a study performed by Huang *et al.* (2011) which was performed on *Cryptosporidium* spp. in wastewater showed that the level of pathogen destruction was directly proportional to the US energy intensity, as a result of the destruction of the oocysts via shattering following the collapse of the cavitation bubbles. A 3-log reduction of pathogenic protozoans was noted with a treatment time of nine minutes at 413 $W.cm^{-2}$. Babu *et al.* (2016) explain that hybrid AOPs, which is the combination of conventional AOPS with ultrasound technology, resulted in superior efficacy of disinfection, with the outcomes being synergistic and additive. This hybrid method of water treatment is simple to operate, environmentally benign and is commercially viable in both small- and large-scale processing plants.

Electron beam technology

Comet Group (2019) states the use of electron beam technology is an energy-efficient alternative to traditional practices used in industry that reduces the demand on harmful chemicals which negatively impact the environment. This type of technology has been widely used since the 1950s for applications such as wire insulation as well as cross-linking of

polyethylene films but has extended to multipurpose applications such as sterilisation in the medical industry, water and wastewater disinfection, welding and food preservation amongst others (Waite *et al.,* 1998).

In this process, high energy electrons come into contact with the material, which react instantaneously and causes the electrons to slow down to energies lower than 50 eV. These slow electrons are then able to interact with the material to produce molecules, electrons and positive ions of excited states within the materials. Electrons aren't able to escape the pull of positive ions formed in materials that have a low dielectric constant which results in a chemical reaction occurring when the electrons are attracted back to the positive ions. This is termed direct radiolysis. Water, a material with a high dielectric constant, causes a reaction referred to as indirect radiolysis, occurs when electrons are able to resist the pull of positive ions, ultimately leaving them able to react with the water molecules and components within the water sample (Waite *et al.,* 1998). This results in the decomposition of pollutants due to the interactions with reactive species (Han *et al.,* 2001).

A study performed by Maruthi *et al.* (2011a) described the four methods of microorganism inactivation through the use of electron beam technology, namely; critical enzyme inactivation, cell wall damage, change in permeability of the cell, and alteration in the colloidal nature of cell protoplasm. Maruthi *et al.* (2011a) denotes the factors that affect the efficacy of this method include the dosage, dose distribution, quality of radiation and exposure patterns. Characteristics of the organism to take into account when using this technology include growth phase, microbial load and sensitivity to treatment. The study concluded that dosages of approximately 3 kGy are effective at producing adequately disinfected water from sewerage water samples, which is fit for use in both industrial and agricultural practices. Maruthi *et al.* (2011b) added that a dosage of 1.5 kGy was sufficient for the destruction of total coliforms from water samples contaminated with faeces to undetectable levels. *Salmonella* and *Shigella* counts were reduced significantly with the application of a 0.75 kGy treatment with an electron beam. The ability of electron beam technology to effectively reduce BOD and COD will ultimately increase water quality (Maruthi *et al.,* 2011b).

This method of disinfection is advantageous as it operates at ambient temperatures and pressures and is not affected by the solid content in the water sample. The greatest advantage of this method is the reduction in dependence on chemicals for purification that could damage and pollute the environment downstream, as well as not producing any radioactive material (International Atomic Energy Agency (IAEA), 2012). A water treatment plant in the Republic of Korea has combined the use of electron beam technology with a biological treatment to treat more than 10 000 m^3 of wastewater per day that is used industrially and in agriculture (IAEA, 2012). Several limitations of this technology have been proposed and these include;

worker safety when working with highly reactive electrons, high set-up and maintenance costs, no long-term operating history of this technology and the limitation of penetration of a few centimetres (Maruthi, *et al.,* 2011b). Another limitation is the challenge of employing this treatment method in a farm-environment.

2.10 Ultraviolet light radiation as a method of irrigation water treatment

2.10.1 Introduction to Ultraviolet light radiation

The use of sunlight to inactivate microorganisms was first discovered in 1877 by Downes and Blunt who noted that the germicidal ability was dependent on dosage, wavelength and microorganism sensitivity (Reed, 2010). Whitby & Scheible (2004) denote that UV radiation and its application in the water treatment industry was first introduced in 1910 and has since gained exponential momentum due to the reliability, efficacy as well as efficiency as a water treatment method. The discovery of UV radiation, however, spanned over three centuries when scientists noted how sunlight was able to change the colour of crystals or chemical-soaked paper (Hockberger, 2002). Consumer demand to lower carbon footprints and lead more environmentally sustainable lives has resulted in investigations into alternative technologies for disinfection purposes to lower dependence on chemicals and other harmful substances (Koutchma, 2009). This pressure from consumers has resulted in the investigation into the use of radiation for disinfection purposes as an alternative to both chemicals and thermal processing. UV radiation falls within the wavelengths of 100 - 400 nm in the electromagnetic spectrum and is divided into four spectral areas (Table 5).

Cutler & Zimmerman (2011) describe vacuum UV as being of the highest energy-containing spectral area of UV. Dohan & Masschelein (1987) denote that this type of UV radiation can be used to produce ozone for use in water treatment via the production of free radicals. The United States Environmental Protection Agency (USEPA) (1999) states that this type of technology is impractical due to the rapid dissipation of this energy over very short distances.

Table 5 The four spectral regions of UV radiation with the corresponding wavelength bands (Dai *et al.,* 2012)

Spectral area	Wavelength band (nm)
Vacuum UV	100-200
UVC	200-280
UVB	280-315
UVA	315-400

UV-A and UV-B radiation are most notoriously associated with skin damage from sun exposure as a result of cell injury. Experiments on fruit flies and other experimental animals that were treated with light within the UV-A and UV-B range, resulted in the formation of tumours with the UV-A rays being the most carcinogenic (Hockberger, 2002). The damaging effects of UV-A and UV-B radiation include that of photo-aging and photo-carcinogenesis. According to Gabros & Zito (2019), the ozone layer around the Earth is able to absorb all of the UV-C radiation from the sun, 90% of UV-B and minimal amounts of UV-A radiation. The reduction in the ozone layer, due to carbofluoro-carbons and other chemicals, results in the penetration of harmful rays causing damage to human, animals and vegetation on Earth. UV-A radiation is therefore, responsible for the harmful effects of the sun as it is able to penetrate deep within the skin to produce free-radical oxygen species, resulting in damaged cell DNA. UV-B, however, is responsible for causing sunburn which could lead to non-melanoma skin cancers but plays a role in the synthesis of vitamin D within the body, whereas UV-A is responsible for the tanning of human skin (Cutler & Zimmerman, 2011, Gabros & Zito, 2019). Broad spectrum sunscreens are able to absorb both UV-B and UV-A radiation (Gabros & Zito, 2019).

The UV-C portion of the spectrum is strongly absorbed by the nucleic acids of the microorganisms, and therefore, has application in the inactivation of pathogenic and indicator organisms in both food and water samples. So much so, that at 253.7 nm, a peak can be identified for its germicidal ability, formally known as the germicidal spectrum and therefore, UV-C radiation is often referred to as UV-G (Dai *et al.,* 2012). Most UV-C radiation studies have been performed *in vitro* and *ex vivo* and have shown great success in the germicidal ability over the past 100 years, however, there is a gap within *in vivo* animal studies as well as clinical studies (Dai *et al.,* 2012). Gayán *et al.* (2012) explain that UV-C radiation is effective for the disinfection across most Gram-positive organisms, yeasts, spores, viruses and moulds. Gram-negative organisms show an even less resistance than Gram-positive organisms, to this treatment method, due to structural differences. However, the efficacy of disinfection varies across dose and exposure time requirements for different groups of microorganisms. The resistances and lethal dosages vary widely amongst different organisms and strain-to-strain. There are three considerations to take into account for quality assurance of this treatment method, the first includes that extensive knowledge must be obtained regarding the resistance of the microorganisms under investigation to the UV light, which will aid in the decision making process for dosage requirements. Secondly, surveillance and inspection must be conducted throughout the application of UV treatment to ensure sample meets specifications, which includes the use of a calibrated UV sensor. Lastly, a broad knowledge of

the available commercial UV equipment should be ensured to ascertain which is best suited for the application (Sommer *et al.,* 2008).

Sommer *et al.* (2008) describes the two types of UV radiation that are employed for water disinfection purposes. The first involves using lamps with low-pressure that produce emissions of a quasi-monochromatic nature at a wavelength of 253.7 nm. Quasi-monochromatic light can be defined as a source that provides radiation that is confined to a specific wavelength or narrow waveband as well as behaving similarly to an ideal monochromatic light (Sharma, 2006). Secondly, using lamps of medium-pressure which emit light of a polychromatic nature. Due to the broader wavelength range used in medium-pressure radiation, it is difficult to ascertain the exact cause of inactivation due to multiple structural changes caused by the radiation (Sommer *et al.,* 2008).

2.10.2 Basic principles and equipment required for UV radiation

Three types of UV equipment designs used for disinfection purposes dominate the commercial industry, namely; open-channel systems, closed channel systems or closed-pipe systems. The most commonly used design for wastewater treatment are the open channel reactors, whereas closed-pipe systems are employed for the treatment of drinking water (Bolton & Cotton, 2008, Linhaber & Jaramillo, 2012). In both closed-pipe and channel systems, UV lamps are placed inside of covered UV-transmitting quartz that is submerged in the flow of the water (Linhaber & Jaramillo, 2012). The function of this quartz sleeve is to protect the lamps from damage as well as maintaining a constant temperature for effective lamp functioning (International Water Guard (IWG), 2002). The quartz ensures sufficient UV-C transmission, as regular glass has shown to block a proportion of radiation in this wavelength range. The use of medium-pressure (MP), low-pressure (LP) and low-pressure high output (LPHO) mercury lamps are most regularly employed for disinfection purposes in water and food industries, with their differences based on the vapour pressure at which they operate, as well as wavelength bands emitted. Mercury is the metal of choice for this application due to the low vapour pressure and the ability to activate with ease as compared to other metals (Koutchuma, 2009). Pulsed UV radiation utilises pulsed xenon lamps which is considered to be a rapid and effective method of microbial inactivation due to the intense, short pulses across a wide wavelength spectrum (Gayán *et al.,* 2014). Low-pressure lamps emit single or narrow wavelength bands, targeted at the wavelength of maximum DNA absorbance, located at 253.7 nm on the electromagnetic spectrum (Kowalski, 2009). In open-channel systems, these lamps are placed perpendicularly or in parallel to the flow (Bolton & Cotton, 2008). Medium-pressure lamps emit a wider polychromatic spectrum of wavelengths between 200 and 600 nm, with only between 15 – 23% of emissions at the maximal DNA absorbance wavelength of 253.7

nm (Kowalski, 2009). According to Zimmer & Slawson (2002), the increased pressure and intensity of this radiation from a MP lamp reduces the number of lamps that are required. The lamps are always placed in a manner to provide the highest intensity. As the UV light propagates through the sample, it interacts with particles within the water resulting in it being reflected, absorbed, refracted or scattered. This interaction with matter determines the efficacy of microorganism inactivation (Koutchuma, 2009).

2.10.3 Mechanism of inactivation by UV radiation

The mechanism of microbial inactivation by UV radiation is as a result of lethal DNA damage (Reed, 2010). The nucleotides and proteins of a microbial cell are the only components that are able to absorb UV radiation in significant amounts in this specific wavelength range (Bolton & Cotton, 2008). Figure 3 shows the relationship between absorbance of radiation by nucleotides and proteins.

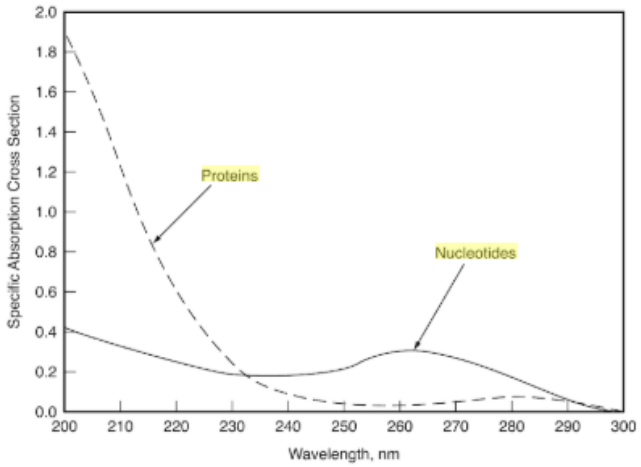

Figure 3 Nucleotide and protein peaks at various wavelengths measured using a spectrophotometer (Bolton & Cotton, 2008)

Proteins are responsible for most of the absorption of UV radiation below 230 nm, whereas nucleotides dominate absorbance above 230 nm. Due to the fact that water is able to absorb light below 230 nm, higher dosages of UV radiation will be required for disinfection at lower wavelengths. Therefore, maximum disinfection efficacy can be obtained by utilising wavelengths higher than 230 nm, as water absorbance at these wavelengths is decreased, and the DNA of an organism can be effectively targeted. This results in the requirement for higher UV doses to disrupt protein activity within microorganisms as compared to doses required for DNA and RNA disruption (Bolton & Cotton, 2008). DNA contains phosphate groups, sugar moieties and either a pyrimidine or purine molecule. Due to the fact that the phosphate and sugar molecules absorb UV radiation below 210 nm, the purine and pyrimidine

molecules are responsible for absorption at higher wavelengths, and are thus targeted (Gayán *et al.,* 2014).

The small increase in absorption at 280 nm by proteins can be attributed to the aromatic amino acids such as tryptophan, tyrosine and phenylalanine, with tryptophan absorbing the strongest at 280 nm. These aromatic compounds; along with pyridines, pyrimidines, and flavins, contain conjugated double bonds that hold two electron pairs. The two molecular bonds occurring within these structures are Sigma (σ) and Pi (π) orbitals. Within conjugated ring structures of these compounds; large, non-localized π-orbitals dominate, which are stable and exhibit a longer wave function than σ-orbitals (Cutler & Zimmerman, 2011). The absorption of the photon energy by these bonds, results in the promotion of the electrons to high energy levels, and leads to the conversion to vibrational energy. This unstable state must return to ground state either via the dissipation of this gained energy or through bond rotations (Cutler & Zimmerman, 2011).

Within RNA, uracil and cytosine are the targets of UV inactivation whereas thymine and cytosine are targeted in DNA. Damage to the DNA occurs following the absorption of high energy photons and the formation of one of six possible photoproducts. These include cyclobutane pyrimidine dimers (CPDs) and to a lesser extent, pyrimidine 6-4 pyrimidones (6-4PPs) as described by Cutler & Zimmerman (2011) and Dai *et al.* (2012). It is interesting to note that pyrimidines are ten times more sensitive to UV energy than purines, therefore, these photoproducts are of primary target (Gayán *et al.,* 2013). Gayán *et al.* (2014) states that CPDs are formed when a pyrimidine molecule absorbs a photon of energy, causing it to covalently bond via the carbon-5 and -6 of an adjacent pyrimidine molecule forming a ring structure. Unstable products such as oxetane and azetidine are produced in the formation of 6-4PP molecules, when the carbon-5 and -6 of a pyrimidine molecule reacts with the fourth carbon on a carbonyl or imino group for the 3' neighbour. Photoproducts include thymine-thymine, uracil-uracil, cytosine-cytosine, cytosine-thymine, uracil-thymine and uracil-cytosine dimers, with the first two dimers requiring the least energy to form. Thymine dimers are produced with the highest yield, and form after the hydrogen bond linkage between bases is lost, resulting in carbon-5 and -6 becoming cross-linked. This ultimately results in the prevention of further transcription and replication, resulting in mutagenesis and ultimately leading to cell death (Reed, 2010, Cutler & Zimmerman, 2011, Gayán *et al.,* 2013). Bolton & Cotton (2008) noted that once a certain number of dimers is formed within microbial cells, DNA replication will be inhibited and this, then, is the primary UV disinfection mechanism.

2.10.4 Factors that influence the efficacy of this treatment method

Absorption of light is defined by Koutchma (2009) as the transformation of photon energy to other energy forms following the interaction with a substance. The higher the level of absorbance by substances, other than the target organism within the sample, the lower the dose delivery for the intended microbial inactivation. Hassen *et al.* (1999) and Gayán *et al.* (2011) describe the factors that affect the efficacy of this treatment method in disinfection applications as lamp ageing, turbidity, TSS, COD, and the UV transmittance (UVT %). Liu (2005) describes that temperature and pH have no direct effect on disinfection efficacy. However, this statement may come as a contradiction to a study performed by Gayán *et al.* (2012) on the effect of UV radiation on *Salmonella* spp. in combination with mild heat treatments (UV-H). This study showed that temperatures of 50 – 60°C increased the lethality of the radiation by between 1.18 ± 0.06 and 6.62 $\log_{10,}$ respectively. The inclusion of heat proved successful in comparison to the application of UV radiation at room temperature which hardly achieved a reduction of 0.64 ± 0.08 $\log_{10}$ for the same *Salmonella* strains. The mechanism of increased inactivation with this combination method is as a result of bacterial cell envelope damage due to the applied heat. The pH of the sample may play a role in negatively impacting treatment, as an increased pH may result in dissolved metals precipitating out of solution, leading to an increase in turbidity, which results in decreased inactivation efficacy (Farrell *et al.,* 2018). The COD provides an indication of the level of organic pollution within a water sample. Total organic carbon and phenols contribute to the absorption coefficient of water. Since both organic and inorganic compounds are able to absorb UV light in water, it is noted that both COD and TDS are inversely proportional to the UVT % (Olivier, 2015). Suspended solids, according to Abdul-Halim & Davey (2016), reduce the efficacy of this treatment method either by shielding the microorganisms from the UV radiation or by absorbing the UV radiation, thereby, reducing the energy available for microbial inactivation. When UV light is absorbed by suspended solids, it is no longer available for the inactivation of microorganisms and should be taken into account when determining the dosage requirements (Liu, 2005). Water that has a high turbidity impacts disinfection efficiency by lowering the UVT %, whereas lower turbidity improves the disinfection capacity by increasing the UVT %. This is due to the fact that, much alike suspended solids, microorganism aggregates can be enclosed within the particulates in the turbid samples, which increases the microorganisms resistance to UV penetration dramatically (Farrell *et al.*, 2018). Liu (2005) reported that an increase in turbidity from 1 to 10 NTU would reduce the average dosage by between 5% and 33%. Olivier (2015) states that the age of the lamp used in the UV system may affect the disinfection efficiency as a result of microorganism's potential to repair themselves following treatment with older lamps. High levels of dissolved substances such as

iron, calcium and hydrogen sulphide negatively impact this treatment method by the formation of a thin coat around the unit, which decreases the UV intensity and therefore reduces output power (USEPA, 1999, IWG, 2002).

The UV dose can be expressed as the product of intensity and exposure time ($J.m^{-2}$ or $mJ.cm^{-2}$ or $mW.s.cm^{-2}$). Intensity is affected by both water quality and the output of the lamps within the system. Intensity decreases with an increase in turbidity and suspended solids as the UV light becomes obstructed or absorbed by other particulates in the water. Optimal disinfection results may be achieved by using a pre-filtration step to remove these particulates within the water (IWG, 2002). The output of the lamp can be controlled with effective cleaning of the quartz sleeve that contains it. The exposure time is directly related to the flow rate of water passing through the system. The IWG (2002) states that a longer period of UV application, the higher the dose applied becomes. Equation 4 denotes the method of calculating the average intensity of the UV light (Hallmich & Gehr, 2010). This value is required to calculate the required time of exposure to UV radiation, which can be seen in Equation 5.

$$I_{\text{avg},\lambda\,(mW.cm^{-2})} = I_0\lambda \left[\frac{1-e^{d\ln(\text{UVT}(\lambda))}}{-d\ln(\text{UVT}(\lambda))}\right] \qquad \ldots. [4]$$

In this equation, $I_{(\text{avg},\lambda\,.)}$ refers to the average UV light intensity over sample depth (*d);* UVT (λ) is the UV transmission of the sample measured at a wavelength of λ (254 nm), using an optical path length of 1 cm, and $I_0(\lambda)$ refers to the UV light intensity measured at the sample surface. The value obtained is then inserted into Equation 5, which provides the exposure time required to achieve a specific dose (Hallmich & Gehr, 2010).

$$Desired\ dose\ (mJ.cm^{-2}) = Average\ intensity\ (mW.cm^{-2}) \times Exposure\ time\ (s) \ldots [5]$$

Cutler & Zimmerman (2011) and Gayán *et al.* (2014) describe the use of UV radiation for disinfection purposes as exhibiting first order kinetics. This can be explained as the amount of irradiance equals the amount of product (which in this case is inactivated microorganisms) within a given period of time. Simply put, “one hit kinetics” describes that the inactivation of microorganisms is due to a single event, such as one photon reacting per organism, where each organism has an equal probability of death. However, this is not always the case as multiple photons might need to hit one target to overcome the capability of the organism to repair its DNA, as well as variations in resistances in environmental strains resulting in higher dosage requirements.

2.10.5 Ultraviolet dosage requirements

The UV dose can be defined as the measurement of the energy per unit area that falls upon a surface and is most commonly expressed as $mJ.cm^{-2}$ (Johnson *et al.,* 2010). Gayán *et al.*

(2014) explains that dosage requirements are dependent on microbial sensitivities and may be attributed to intrinsic and extrinsic factors. Cell wall structure and thickness, the presence of UV absorbing proteins, cell and genome size and most importantly, the ability of the microorganism to repair the genetic damage are amongst the many intrinsic factors that determine the sensitivity to UV light (Koutchuma, 2009). Bolton & Cotton (2008) describe that UV sensitivity differences between different organisms result in difficulty in determining dosage requirements to achieve a predetermined log reduction in mixed microbial populations. To overcome this limitation, UV dose-response curves have been developed, using collimated beam tests, to effectively treat samples to achieve certain log reduction, taking resistant microorganisms into consideration. As a general rule of thumb, as described by Gayán *et al.* (2011), Gram-negative microorganisms are more sensitive to UV radiation than Gram-positive organisms, yeasts, spores, moulds and viruses. Therefore, it is of great importance to determine the microbial type and load present within the water source in order to apply an effective dose to achieve the required result. Hoffman *et al.* (2004) indicates that Adenovirus-40 requires a dosage of 120 $mJ.cm^{-2}$ to achieve a 4-log reduction, which is of importance due to the fact that this virus has been detected worldwide within sewerage systems. One should always select a dosage that will inactivate even the most resistant organism present in the water sample.

Many conflicting reports have been made regarding the doses required for the inactivation of protozoan pathogens such as *Cryptosporidium parvum* and *Giardia lamblia*. The USEPA (1999) reports that for a 2-3 log reduction of these organisms, dosages of 41 $mJ.cm^{-2}$ and 63.3 $mJ.cm^{-2}$ are required for *Cryptosporidium parvum* and *Giardia lamblia,* respectively, and states that these organisms are highly resistant to treatment. Contrastingly, to achieve a 3-log reduction in both *C. parvum* and *G. lamblia,* UV doses of <5 $mJ.cm^{-2}$ and <20 $mJ.cm^{-2}$ respectively have been suggested, and is has been noted that these organisms are highly susceptible to UV treatment (Campbell *et al.,* 2002, Amoah *et al.,* 2005, Hijnen *et al*., 2006). This is incredibly significant as chemical disinfection techniques, such as the use of chlorine, have shown limited application for the inactivation of protozoan (oo)cysts (Hijnen *et al.,* 2006). Table 6 provides a summary of the estimated dosages required to achieve a 1-log reduction (D_{10}) in specified microorganisms within certain groups.

Table 6 Dosage requirements for different microorganisms groups as well as specific organisms treated with UV radiation at 253.7 nm to achieve a 1-log reduction (Amoah *et al.,* 2005, Koutchuma, 2009, Gayán *et al.,* 2014, USEPA, 1999)

Microorganism group	Average UV dose required ($mJ.cm^{-2}$)
Enterobacteriaceae	2.0 – 8.0
E. coli O157: H7	3.5
Salmonella typhi	1.9
Others from Bacteria domain	
Listeria monocytogenes	2.6
Pseudomonas aeruginosa (ATCC 10145)	4.6
Staphylococcus aureus (ATCC 6538)	5.6
Cocci and micrococci	1.5 – 20.0
Enterococcus faecalis	4.2
Enterococcus faecium	3.4
Spore formers	4.0 – 30.0
Clostridium perfringens spores	16.7
Bacillus subtilis spores	16.9
Protozoan pathogens	5 – 120.0
Cryptosporidium parvum	<5
Giardia lamblia	<10
Yeast	2.3 – 8.0
Fungi	30.0 - 300
Algae	300.0 – 600.0
Viruses	
PRD-1 (Phage) – S. typhimurium	9.9
MS-2 (Phage) – E. coli 15597	20

Gayán *et al.* (2011) explain that the UV dose that is absorbed by microorganisms proves difficult to accurately measure, which is highly dependent on the exposure time and radiation. The magnitude of this dependence is determined by the flow of the water through the equipment. It has been reported that a turbulent flow will increase the efficiency of treatment as compared to laminar flow (Gayán *et al.,* 2011). Gayán *et al.* (2014) state that larger sized cells have a greater resistance to UV radiation due to an increased probability of the energy-

containing photons being absorbed by other components within the cell, and therefore, not causing damage to the DNA. This can be used to explain the much higher dosage requirements from larger cells such as moulds and yeasts as compared to bacteria. Olivier (2015) states that complex samples such as irrigation water that contains a broad microbial population with various pathogenic strains may reduce the efficacy of this treatment method, and needs to be taken into account when evaluating the disinfection efficacy. The USEPA (1999) suggests that a dose of between 21-36 $mJ.cm^{-2}$ should be sufficient in the inactivation of bacterial and viral pathogens depending on water quality parameters such as turbidity and COD.

2.10.6 Microbial resistance and photo-repair mechanisms

A combination of intrinsic and extrinsic parameters are responsible for the ability of microorganisms to resist inactivation due to UV radiation. Dai *et al.* (2012) state that fast multiplication rates allow microorganisms to rapidly adapt to environmental stresses, which result in favourable competitive advantages that will eventually spread throughout the entire population. Gayán *et al.* (2014) described that the main parameters that determine microbial resistances to UV treatment are wavelength and UV dosages. A higher lethal efficiency is achieved at wavelengths that are close to the absorption peaks of DNA. Intrinsic factors include the species and strain of the microorganism. Increased resistances in bacterial spores can be attributed to their relatively dehydrated state and the thick spore protein-coating preventing the formation of pyrimidine dimers (Riesenman & Nicholson, 2000). A study performed by Sommer *et al*. (2000) noted that widely-diverse doses are required for enteropathogenic *E. coli* inactivation, which exhibited 13 different D_{UV} values for the same log reduction. Gayán *et al.* (2014) state that, in general, pathogenic bacterial strains are more resistant to UV radiation than non-pathogenic strains. A study performed by Gayán *et al.* (2012) on the effect of UV radiation on different *Salmonella* serovars with regard to their individual resistances, noted that *Salmonella typhimurium* STCC 878 was the most resistant strain, requiring 18.03 $mJ.cm^{-2}$ to achieve a 4-log reduction. The most resistant microorganism to date is that of *Deinococcus radiodurans,* an extremophilic bacterium, which requires a dose of between 19.7 and 145 $mJ.cm^{-2}$ for inactivation (Koutchuma, 2009). According to a study performed by Dai *et al.* (2008), excessive repetition of UV radiation may result in resistances developed by microorganisms. Kalisvaart (2004) explain there are two mechanisms of DNA repair, the first is that which requires light and therefore, termed photoreactivation. The other is that which does not, namely, dark-repair. The use of either mechanism is dependent on the biological organisation level as well as the kind of damage inflicted. The ability of microorganisms to repair their DNA after replication errors or endogenous and exogenous DNA-damaging agents is due to various enzymatic repair pathways.

Mismatch repair (MMR), base excision repair (BER) as well as nucleotide excision repair (NER) are the repair mechanisms that have been adopted to repair damage to DNA without the dependence of light. These are all based on the principle of the splicing out of the damaged region and the insertion of new bases and ligation of the damaged pieces (Friedberg, 2003, Rastogi *et al.,* 2010, Gayán *et al.,* 2013). Nucleotide excision repair is one of the most versatile and flexible repair mechanisms found in organisms and is highly conserved in eukaryotes (Rastogi *et al.,* 2010). Two modes of NER have been established; repair of damage over an entire genome and repair of transcription-blocking lesions that are present in transcribed DNA strands (De Laat *et al.,* 1999). Nucleotide excision repair involves the direct removal of the nucleotides containing lesions, and can remove a broad spectrum of lesions through a variety of cascade reactions carried out by the U_{VR}ABC exinuclease enzyme. This excision method removes large lesions such as CPDs, 6-4PPs as well as some forms of oxidative damage (Rastogi *et al.,* 2010).

Base excision repair (BER) arises from hydrolytic deamination and involves the removal of the damaged base, resulting in an apurinic or apyrimidinic site that is subsequently removed (De Laat *et al.,* 1999). This repair mechanism is dependent on glycosylase enzymes for the recognition of specific lesions within the nucleotide bases, which are then removed. The efficiency and specificity of this repair process is dependent on the different forms of this enzyme which are responsible for the removal of differently affected bases (Rastogi *et al.,* 2010). Mismatch repair (MMR) mechanisms are used to repair mistakes made in the replication processes, performed by DNA polymerase enzymes. This is achieved by a group of proteins that detect and correct base errors which occurs when bases are incorrectly inserted, deleted and incorporated within the DNA strand.

Water is treated within enclosed treatment systems, but is often pumped into holding tanks or drained into surface waters before use in irrigation systems. This exposure to sunlight may affect the quality of the water post-treatment, where microorganisms that have been previously deactivated are able to repair themselves and cause an increase in pathogenicity (Quek & Hu, 2013). Photoreactivation is a light-mediated enzymatic repair mechanism that involves the action of the photolyase enzyme, which binds to the CPD lesions for removal. The photolyase enzyme contains FAD as a cofactor and a chromophore as a light harvesting antenna (Thoma, 1999). Together with the chromophore, which is responsible for the conversion of light energy to chemical energy, the enzyme directly reverts the damaged DNA to its undamaged form (Clancy, 2008). In the case of CPDs, this enzyme functions by binding to the DNA, flipping the pyrimidine dimer out of the DNA strand and into a hole that contains the FAD molecule. Light-initiated electron transfer reactions cause the cyclobutane ring to be split. Other enzymes required for photoreactivation include endonuclease, polymerase and

ligase (Kalisvaart, 2004). This type of repair is not limited to bacteria, but extends to algae, protozoa, vertebrates and mammals (Das, 2001).

Kalisvaart (2004) states that for the initiation of the photoreactivation process, exposure to light between 310 and 480 nm is required for a few minutes, depending on the organism. Organisms have developed such diverse repair mechanisms to environmental stresses that below the lethal UV dose, the potentially lethal effects of this treatment method can be avoided. In water treatment systems, often indicator organisms are able to reactivate easier than the pathogens, which results in an overestimation of the pathogen numbers (Zimmer & Slawson, 2002). Zimmer (2003) noted that pathogenic *E. coli* O157:H7 was able to undergo dark repair as well as photoreactivation when treated with LP lamps, but showed undetectable repair mechanisms when treated with MP lamps. Quek & Hu (2013) state the possible reasons for this include increased dimer formation, damage to critical replication enzymes or amino acids and damage to the photolyase enzyme occur from MP UV radiation as compared to monochromatic light used in LP UV radiation. The damage to the photolyase was reported in a study by Quek & Hu (2008) were it was determined that MP UV radiation caused the oxidation of the FAD molecule within the enzyme. A study performed by Oguma *et al.* (2005) investigated the effect of various wavelengths using MP UV radiation to determine dimer repair ability *in vivo*. It was noted that dimer repair was not affected by single wavelengths and therefore, the action of simultaneous exposure to multiple wavelengths enabled for photoreactivation suppression (Oguma *et al.,* 2005). Quek & Hu (2013) performed a study on photolyase to determine the effect of radiation, prior to the UV treatment, on the enzyme activity at different wavelengths. This study utilised one photolyase enzyme which was exposed to UV radiation and one that was not. A decreased dimer repair rate was noted (between 8 and 25%) in the exposed photolyase experiment as compared to the unexposed enzyme experiment. For applications such as irrigation systems, it is important to take the contribution of natural sunlight into account when determining photoreactivation ability. Guo *et al.* (2009) performed a study in which a lamp emitting a spectrum representing that of natural sunlight was utilised to determine the ability of sunlight to initiate photoreactivation post-treatment. It was reported that the *E. coli* strain *CGMCC 1.3373* showed 50% and 20% photoreactivation after initial UV treatment using 5 $mJ.cm^{-2}$ using LP and MP UV systems, respectively. This provides an indication that, at low UV doses and more specifically the use of LP UV systems, sunlight may play a significant role in microbial repair (Guo *et al.,* 2009). Areas where sunlight is plentiful and temperatures between 23-37 °C are a cause for concern for photoreactivation (Quek & Hu, 2008).

Furthermore, it was established that higher wavelengths result in increased ability for photolyase repair as compared to lower wavelengths. This could be attributed to photolyase

absorbing strongly below 300 nm, with maximum absorbance peaks noted at 280 m and emission peaks at 266 nm and 365 nm, and therefore, damage to the enzyme could alters its repair mechanisms. In the study performed by Quek & Hu (2013), wavelengths of 254 nm exhibited the lowest dimer repair rate, as compared to wavelengths of 365 nm which showed to have increased dimer repair rates. This may be due to the fact that the enzyme utilises light energy between 300 and 500 nm to perform its repair and therefore, wavelengths of 365 nm are likely to aid in the process of photoreactivation (Quek & Hu, 2013). The study concluded by noting that shorter wavelengths have a greater ability at preventing dimer repair, however, filtered MP UV radiation at 254 nm has a greater effect than LP UV radiation at 254 nm due to the filtered MP UV including peaks at 266 nm (Quek & Hu, 2013).

2.10.7 Advantages and limitations of this treatment method

Several advantages of the use of UV technology for disinfection purposes have been proposed by Bolton & Cotton (2008). These include that this treatment method is effective at not only inactivating bacteria, but extremely effective for the disinfection of *Cryptosporidium* and *Giardia* spp. The equipment is relatively simple to use, and can be adjusted according to site-specific water quality and target organisms. This is a rapid treatment method, requiring only a few seconds as compared to upwards of 30 minutes for certain chemicals. Possibly the largest advantages of this treatment method include that there are no DBPs formed and therefore, can be considered to exert a small footprint on the environment as well as no residuals remain in the water (Bolton & Cotton, 2008).

Disinfection residuals are maintained in chlorine-treatment systems via the addition of chlorine or chloramines after initial treatment, this however, is not possible for UV treatment systems and therefore, can be seen as a limitation (Bolton & Cotton, 2008). Difficulties in the monitoring of the UV dosages result in reliance on sensor readings or water flow rates and thereby, providing some level of inaccuracy. Mercury lamps provide some level of hazard due to possibility of breakages. Highly turbid water samples may result in decreased water treatment efficiency and therefore, the requirement for pre-treatment methods such as filtration becomes essential (IWG, 2002). Lastly, limited water disinfection can occur when the lamp is warming up or if there are power interruptions and therefore, would affect water treatment capacity (Bolton & Cotton, 2008). These limitations could be overcome by using a solar power installation or the utilisation of a battery back-up (Bolton & Cotton, 2008).

It can, therefore, be concluded that the use of UV technology is an attractive and effective treatment method for water. The prevention of DBPs, the reduction of requirements on harmful chemicals further increase the attractiveness of this method. This treatment method has proven to be successful as a treatment method for water, even being able to

produce water of a high enough standard for drinking. Photoreactivation is of concern in surface waters that are exposed to sunlight as it provides the radiation required for organisms to repair and reduce the expectant disinfection result. This can be overcome by utilising MP UV lamps as well as dosages that would completely inactivate the photolyase enzyme. This treatment method should be utilised in combination with a pre-treatment step in the case of highly turbid water samples for effective treatment.

2.11 Concluding remarks

The promotion of healthy eating has resulted in an increased consumption of fresh produce around the world (Alegbeleye *et al.,* 2018). Due to the fact that fresh produce is mostly eaten raw or after minimal processing, there is a potential health risk for the consumption of pathogens (Alegbeleye *et al.,* 2018). Contamination of fresh produce has frequently been associated with irrigation water of poor microbial quality (Pachepsky *et al.,* 2011). Microorganism carry-over from irrigation water to crop is a major concern in the case of food safety, since it can result in foodborne outbreaks (Huisamen, 2012). Surface waters are the preferred source of water supply for irrigation purposes in South Africa due to the fact that it is more economically feasible to obtain than ground waters, however, the risk of contamination of surface waters as compared to ground waters is substantially higher (Singh, 2013, Maree *et al.,* 2016). The microbial and physico-chemical characteristics of surface waters vary widely. This can be attributed to a number of factors including upstream commercial and recreational activities, resulting in the contamination of water (Sousa *et al.,* 2007). These factors need to be taken into account when determining the appropriate treatment to ensure a consistently safe water supply. Continued investigations into the efficacy of different water treatment methods are imperative to make accurate recommendations for optimised water disinfection, and to ensure consumer safety.

Conventional water treatment methods that have been used for centuries include those of chemical or physical treatment methods. Chemical disinfection includes the use of chlorine, peracetic acid, hydrogen peroxide and ozone, amongst others. High levels of organic matter in the water may dramatically decrease the treatment efficacy, and therefore, a filtration pre-treatment step is required. Further limitations in the use of chemicals for water treatment include the formation of DBPs, unwanted odours and tastes, microbial resistances to chemicals as well as the negative impact on the downstream environment (Solomon *et al.,* 1998, Kumar & Pandit, 2013, Giddey, 2015).

Filtration methods are used to physically remove contaminants from the water source. This is often seen as a pre-treatment step that should be used in conjunction with a chemical or photochemical treatment, as well as constant need for maintenance and back-washing. The

efficacy of physical and chemical treatment methods are dependent on a number factors which includes; pH, dissolved solids as well as the turbidity of the water supply (Momba *et al.,* 2008).

Photochemical methods of water treatment include using advanced oxidation processes, electron beam technology and Ultraviolet radiation to disinfect water. These methods were introduced as the need for a sustainable and environmentally friendly method of water treatment became imperative (Galvéz & Rodrígues, 2010). Advanced oxidation processes and electron beam technology have high treatment set-up and maintenance costs, but prove to be an effective water treatment method, nonetheless (Maruthi, *et al.,* 2011b).

Ultraviolet radiation is now a well-established method of disinfection and is routinely used in multiple sectors. As with all treatment methods, there are both advantages and limitations of this treatment method. Short-contact time, ease of operating and limited microbial resistance are possibly the main advantages of UV radiation. Ultraviolet radiation is effective for the treatment of organisms such as *Cryptosporidium* and *Giardia* spp. which have shown resistances to chemical treatments such as chlorine (Bolton & Cotton, 2008). This method of treatment does not result in the formation of DBPs, however, as there are no residuals that remain in the water (as there is with chemical treatments), the chance of recontamination downstream is a possibility. Photoreactivation of microorganisms is another limitation of UV radiation, however, the use of MP lamps and higher dosages are effective measures to combat this possibility.

The efficacy of UV radiation has routinely been tested at laboratory-scale, using LP and/or MP lamps and most often using laboratory-cultured, reference strains of *E. coli* only (Zimmer-Thomas *et al.,* 2007). The need for investigations regarding efficacy of this treatment with the goal of upscaling to farm-level treatment is imperative to understand how effective this treatment would be against naturally occurring microbial populations.

Knowledge gaps also exist in South Africa regarding the efficacy of UV radiation on the disinfection of irrigation water containing food pathogens such as *L. monocytogenes* and *Salmonella* spp. This could be attributed to the fact that limits related to these organisms are not specified in the Irrigation Water Guidelines (DWAF, 1996a). These pathogens are frequently present in surface waters, and therefore, might pose a significant risk to irrigation water safety. They do, however, often go undetected as there is no legislative pressure to test for them.

One way in which this potential threat to irrigation water safety can be addressed is by investigating how these pathogens respond to UV light in water with different physico-chemical profiles. Optimising UV radiation processes under varying conditions is essential to improve disinfection efficacy and address any potential threat to irrigation water safety.

Discrepancies in literature exist with regard to specific UV dose requirements. These, can be attributed to variations in physico-chemical characteristics of the surface waters investigated, as well as a lack of insight into environmental pressures that might cause genetic mutations and increased resistances of specific strains and the variety of microbial populations naturally present in the water that varies over time (USEPA, 1999, Campbell *et al.,* 2002, Amoah *et al.,* 2005, Hijnen *et al*., 2006, Gayán *et al.,* 2011, Gayán *et al.,* 2014). Therefore, the current study was performed in order to address some of these knowledge gaps, and provide recommendations to those treating irrigation water with UV radiation technologies.

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Chapter 3

INVESTIGATING THE DISINFECTION EFFICACY OF LOW-PRESSURE ULTRAVIOLET RADIATION ON IRRIGATION WATER SOURCES, AND THE IMPACT OF WATER QUALITY ON TREATMENT

Abstract

The disinfection ability of Ultraviolet (UV) radiation was tested against four selected rivers used for irrigation purposes. Microbial and physico-chemical tests were performed on water samples to establish a profile of the four rivers. Microbial tests included the quantification of *Escherichia coli (E. coli), Enterobacteriaceae,* Heterotrophic Plate Counts (HPC), and the presence of *Listeria monocytogenes (L. monocytogenes)* and *Salmonella* species (spp.). The physico-chemical results aided in the correlation between water quality and treatment efficacy. The four selected rivers were deemed unacceptable for agricultural irrigation without pre-treatment, based on the microbial results obtained. The river water samples were exposed to three doses of low-pressure (LP) UV radiation (20, 40, 60 $mJ.cm^{-2}$). The dosages were applied by exposing the sample to three consecutive doses of 20 $mJ.cm^{-2}$. Microbial populations, and the presence or absence of two selected pathogens, were recorded before UV radiation, and then after each of the respective doses.

The efficacy of this treatment method for the disinfection of river water, was noted in the log reductions of microbial counts after UV radiation was applied. The largest log reduction was reported for the HPC test in the Mosselbank River, where a 5.2 log reduction was noted (1 780 000 $cfu.mL^{-1}$ to none detectable) after 60 $mJ.cm^{-2}$ of UV radiation was applied. Extreme faecal pollution was noted in these rivers, where the highest recorded *E. coli* count of 2.79 x 10^5 $CFU.mL^{-1}$ was noted in the Mosselbank River. Log reductions for *E. coli* counts over the three UV doses, ranged between 2.9 – 4.4 log, 1.3 – 3.6 log, 1.6 – 2.1 log and 2.3 – 3.9 log, for the Mosselbank, Franschhoek, Eerste and Plankenburg Rivers, respectively. Heterotrophic plate count log reductions ranged between 0.9 – 5. 2 log, 1.1 – 4.5 log, 1.3 – 3.2 log and 2.6 – 4.3 log, for the Mosselbank, Franschhoek, Eerste and Plankenburg Rivers, respectively. The log reductions for *Enterobacteriaceae*, for all three UV doses, ranged between 2.9 – 4.1 log, 1.3 – 3.6 log, 1.5 – 2.0 log and 2.1 – 4.2 log for the Mosselbank, Franschhoek, Eerste and Plankenburg Rivers, respectively. No microbial growth was detected on the agar plates at the highest log reduction value for each river. The presence/absence testing of the pathogens, *L. monocytogenes* and *Salmonella* spp. proved that LP UV radiation is effective for the disinfection at laboratory-scale, even after the lowest dose applied (20 $mJ.cm^{-2}$). The significant reduction in microbial loads, as well as the absence of pathogens after UV radiation applied, indicates the efficacy of this treatment method.

Water quality did not impact treatment efficacy, but significantly impacted the exposure times required to deliver a specific dose. Water was deemed safe for use after UV radiation was applied, and met the guidelines (Department of Water Affairs and Forestry (DWAF), 1996) for the irrigation of fresh produce. The doses of LP UV radiation selected proved successful for disinfection.

Introduction

Fresh water sources, according to Warriner *et al.* (2009), are often associated with the presence of waterborne pathogens. The risk of carry-over of these bacteria, viruses and protozoa from irrigation water to crop is a major food safety concern (Huisamen, 2012). Pachepsky *et al.* (2011) states that this risk is higher in developing countries. In the South African context, this could be attributed to the highly polluted rivers, as well as the scarcity of a consistent water supply. The microbial loads present in certain rivers in the Western Cape have consistently exceeded microbial guidelines in the Irrigation Water Guidelines (DWAF, 1996), and are therefore, deemed unfit for irrigation purposes without pre-treatment (Barnes, 2003, Kahn *et al.,* 2015, Britz *et al.,* 2013, Olivier, 2015, Sivhute, 2019). These findings are not only prevalent for the Western Cape, but similar findings have been reported in surface waters across South Africa (Gemmell & Schmidt, 2013, Chigor *et al.,* 2013, Bezuidenhout, 2013).

Microbial guidelines for irrigation water quality, set out by the Department of Water Affairs and Forestry (DWAF) (1996a), state that faecal coliforms present in the water may not exceed 1 000 colony forming units (cfu) per 100 mL. The need for an effective, efficient and economically feasible treatment method is, therefore, required to ensure crop safety. Britz *et al*. (2012) and Britz *et al.* (2013), state that in order to obtain water that is safe for irrigation purposes, a 3 – 4 log reduction of microbial load is suggested for local rivers, considering the level of contamination that has been noted in previous studies.

Ultraviolet (UV) radiation has become an attractive method of water disinfection in recent years. This is due to a shift to using disinfection methods that do not negatively impact the environment (Koutchma, 2009). UV radiation has proven to be successful for the inactivation of a broad range of microorganisms, including parasites such as *Cryptosporidium* and *Giardia* species (spp.), which have shown resistance to chemical disinfection methods, such as chlorine (Hijnen *et al.,* 2006). Residual chemicals present in water after treatment, as well as the formation of carcinogenic disinfection by-products (DBPs) after chlorine use, further increases the attractiveness of UV radiation as a disinfection method (Solomon *et al.,* 1998, Whitby & Scheible, 2004, Momba *et al.,* 2008).

The efficacy of UV radiation has been frequently tested at laboratory-scale, where laboratory-cultured, reference strains of *E. coli* are predominantly used, and most often low-pressure (LP) UV systems (Zimmer-Thomas *et al.,* 2007). Limited research has been performed in South Africa with regard to the presence of pathogens such as *Salmonella* spp. and *L. monocytogenes* in irrigation water, as well as the response of these microorganisms to UV radiation. These pathogens are frequently present in surface waters (Falardeau *et al.,* 2017, Omar & Barnard, 2010), and therefore, might pose a significant risk to irrigation water safety. They do, however, often go undetected as there is no legislative pressure to test for them. Discrepancies exist in literature with regard to specific doses required for inactivation for different microbial groups. This could be attributed to the variations in environmental or clinical sources of the test strain, as well as the possibility of genetic mutations and increased resistances of specific strains and mixed microbial populations (United States Environmental Protection Agency (USEPA), 1999, Campbell *et al.,* 2002, Amoah *et al.,* 2005, Hijnen *et al*., 2006, Gayán *et al.,* 2011, Gayán *et al.,* 2014).

The aim of the research presented in this study was to investigate the efficacy of laboratory-scale LP UV treatment on the microbial loads present in various rivers used for irrigation purposes. The four selected rivers were sampled five times each, and three doses of LP UV radiation were applied. The microbial quality was reported before and after the UV treatment, where counts for *E. coli, Enterobacteriaceae,* HPC, *Salmonella* spp. and *L. monocytogenes* were performed. The water quality and the understanding of the impact of this on treatment efficacy was determined by testing the physico-chemical characteristics. These tests included chemical oxygen demand (COD), alkalinity, pH, total dissolved solids (TDS), total suspended solids (TSS), electrical conductivity (EC), and turbidity before treatment was applied. The first of the two-fold objective of this study was to provide a broad understanding of the microbial and physico-chemical profile of the four selected rivers. The second was to identify how the microorganisms present in these rivers respond to UV radiation. Microbial isolates from river water before and after UV radiation were retained for further analysis in following chapters.

Materials and Methods

General materials and methods

Site selection and sampling method

The first of the four rivers that were selected for this study was the Plankenburg River, Stellenbosch (-33.932712, 18.85174). This river acted as an experimental control as various, previous studies have consistently indicated extremely high microbial loads. The Plankenburg

River joins the Jonkershoek River downstream to form the Eerste River. The Eerste River, Vlottenburg (-33.980080, 18.775718) was selected as it is routinely used for the irrigation of fresh produce and fruit by commercial farmers. The Mosselbank River sampling site in Kraaifontein (-33.819729, 18.703042) was situated downstream of the sewerage treatment works, which, together with the storm water run-off becomes a water source for large-scale commercial farmers. Lastly, the Franschhoek River, Franschhoek (-33.898283, 19.094181) was sampled at the confluence of both the Berg and Stiebeuel Rivers. This river water is used for the irrigation of grapes but also for large-scale commercial tomato famers. All four rivers shared common characteristics, where they were geographically distributed in the Western Cape, used for irrigation purposes and some being notorious for their high microbial loads (Barnes, 2003, Kahn *et al.,* 2015, Britz *et al.,* 2013, Olivier, 2015, Sivhute, 2019).

The South African National Standards (SANS) method 5667-6 for sampling (SANS, 2006) was utilised to obtain water samples from four rivers around the Stellenbosch area. The water was obtained using a sterile 1 L Schott bottle and transported on ice. All microbiological tests were performed within six hours of sampling, and all physico-chemical tests were performed within 24 hours of sampling (APHA, 2005).

Physico-chemical analysis of river water samples

River water was sampled and transported to the laboratory for microbial and physico-chemical analysis. The results were compared to the guidelines provided for water intended for fresh produce irrigation (Table 1).

Table 1 Suggested limits for physio-chemical parameters of irrigation water (DWAF, 1996a)

Water Quality	**Irrigation water**
TDS	260 $mg.m^{-1}$
Turbidity [1]	10 NTU
Alkalinity [2]	< 120 mg.L $CaCO_3^{-1}$
UVT %	Not stipulated
Chemical oxygen demand [3]	< 75 mg $O_2.L^{-1}$
pH	6.5 – 8.4
TSS	50 $mg.m^{-1}$
Electrical Conductivity	40 $mS.m^{-1}$

[1] Not stipulated. The Water Quality Guidelines for Domestic Use (DWAF, 1996c) states that for turbidity values exceeding 10 NTU, the water carries an associated risk of disease, therefore this was used as the guideline limit.

[2] Not stipulated. Industrial Water Guidelines were consulted (DWAF, 1996b). A value of < 120 mg.L $CaCO_3^{-1}$ was selected as these guidelines state little to minor impairment of crop quality.

[3] Not stipulated. The Guidelines for Industrial Use (DWAF, 1996b) were consulted and an acceptable limits for COD in irrigation water is < 75 mg $O_2.L^{-1}$ was accepted.

Total Dissolved Solids

The Total Dissolved Solids (TDS) of the water sample was determined using a (TDS)-3 meter (HM Digital). This handheld meter is used to determine the total amount of mobile charged ions, which is directly proportional to the electrical conductivity of the sample. The meter expresses the reading in parts per million (ppm) which equates to $mg.L^{-1}$. Each sample was analysed in duplicate, after which an average value was obtained.

Turbidity

The turbidity of river water samples was measured using a portable Orion AQ3010 Turbidity Meter (Thermo Scientific, USA). Values were expressed in Nephelometric Turbidity Units (NTU), prior to use, the instrument was calibrated with samples of known turbidity, starting with the standard of 800 NTU. All samples were then analysed in duplicate.

Alkalinity

The alkalinity value of each water sample was obtained by performing a titration according to Standard Methods (APHA, 2005). A solution of 0.1 N H_2SO_4 was prepared and titrated into a beaker containing 50 mL of sample. The titration was performed until a pH of 4.3 was recorded. The volume of H_2SO_4 required was used in a calculation to determine the alkalinity in units of mg.L $CaCO_3^{-1}$.

Ultraviolet Transmission percentage

A Sense T254 UV Transmission percentage (%) Photometer (Berson, Netherlands) was utilised to determine the UVT % of water samples. The procedure provided by the manufacturer was followed, and the photometer was calibrated using distilled water which represented a UVT % of 100%. Analyses was performed in duplicate.

Chemical Oxygen Demand

This is a measure of the quantity of oxygen that is available for consumption by oxidative reactions in a solution. The chemical oxygen demand (COD) is measured photometrically using the Spectroquant NOVA 60 Spectrophotometer (Merck Millipore, South Africa) and expressed in units of mg $O_2.L^{-1}$. River water samples were tested in the range of 10 – 150 mg $O_2.L^{-1}$. Following the standard testing procedure - three millilitres of each sample were added to a standardised test vial, which contained the required reagents, and vortexed. Samples then were allowed to digest at 148°C for 2 hours in a Spectroquant TR 420 thermal reactor (Hach, USA). The samples were allowed to cool following digestion before COD values were measured photometrically, (Merck Millipore, South Africa), in duplicate.

pH

Using a portable pH meter (WTW, Germany), according to the instructions of the manufacturer, duplicate pH readings for each river sample were obtained. The instrument was calibrated before use using standard pH solutions (using a three-point calibration; pH 7, pH 4 and pH 10).

Total Suspended Solids

The Standards Methods (APHA, 2005) were consulted to obtain instructions for the measurements of total suspended solids (TSS). The TSS was determined by filtering an amount of river water sample through Munktell glass fibre 0.6 µM filter paper (Lasec, South Africa), which was then placed in a crucible at 105°C for 2 hours. The weights of the crucibles were recorded before and after filtration with river water. Cooling was performed in a desiccator for 30 minutes after heating. The respective calculation was performed to determine the values for TSS expressed in units of $mg.L^{-1}$. All tests were performed in duplicate.

Electrical Conductivity

The quantity of the dissolved salts in the river samples were determined by using a portable HI 8733 conductivity meter (Hanna Instruments, USA). The instrument was calibrated according to the manufacturer's instructions. Samples were then measured in duplicate and an average value obtained.

Microbiological analysis of water samples

Following sampling at each river, samples were analysed for the presence of indicators and pathogens such as *E. coli, Salmonella* spp., *L. monocytogenes* and *Enterobacteriaceae*. The HPC was also determined. For the enumeration of *Enterobacteriaceae*, *E. coli* and HPC a dilution series (10^{-1}-10^{-6}) was prepared for each river water sample in test tubes containing sterile buffered peptone water (BPW) (Oxoid, South Africa) following the standard procedures in SANS method 6887-1 (SANS, 1999). Microbial analysis of these samples was further conducted by using the appropriate standard pour or streak plate techniques as discussed in the following section.

Heterotrophic Plate Count enumeration

The Heterotrophic Plate Count (HPC) was determined using Plate Count Agar (PCA) (Oxoid, South Africa) according to the SANS method 4833-1 (SANS, 2007). Duplicate plates for all dilutions and doses were prepared using the standard pour plate technique and the straw-coloured colonies were counted (in the range of 10-300) following inverse incubation at 30°C for 72 hours.

Escherichia coli *identification and enumeration*

The presence and number of *E. coli* was determined using Brilliance Coliform/ *E. coli* Selective Agar (Oxoid, South Africa) following instructions provided by the International Standards Organisation (ISO) 16654 Method (2001). Duplicate plates were prepared for all doses and dilutions and the standard pour plate technique was used. A presumptive positive result for *E. coli* is represented by purple colonies on this agar after incubation at 37°C for 24 hours. The colonies in the range of 10 and 300 were recorded.

Enterobacteriaceae *detection and enumeration*

Enterobacteriaceae counts were determined using Violet Red Bile Glucose (VRBG) Agar (Oxoid, South Africa), and the pour plate technique on duplicate plates for all dilutions and treatments according to the ISO Method 21528-2 (ISO, 2017). Following incubation at 37°C for 24 hours, pink coloured colonies in the range of 10 and 300 were counted.

Salmonella *species identification*

Salmonella spp. identification was based on presence/absence testing using selective media and therefore, no counts were performed. Two selective agars were used, namely, Xylose Lysine Deoxycholate Agar (XLD) and Hektoen Enteric Agar (Oxoid, South Africa), following standard enrichment methodology described in SANS method 19250 (SANS, 2011b). The 25 mL water samples before and after each UV dose were first incubated in 225 mL sterile buffered peptone water for 24 hours at 35°C. Following this incubation, 0.1 mL was then transferred to 10 mL of autoclaved Rappaport Vassiliadis (RV) broth (Oxoid, South Africa), vortexed, and incubated at 42°C for a further 24 hours. Using a sterile loop, each sample was then streaked onto XLD and Hektoen Enteric Agar plates in duplicate. These plates were incubated at 35 - 37°C for 24 hours. Red colonies with black centres were considered as indicative of a presumptive positive result for *Salmonella* spp. on XLD Agar and blue-green coloured colonies with or without black centres indicated *Salmonella* spp. on Hektoen Enteric Agar.

Listeria monocytogenes *identification*

The presence of *L. monocytogenes* was determined by streaking Rapid'*L.mono* Agar Plates with water samples that had been enriched for 24 hours at 30°C in Half-Fraser Enrichment Broth and a *Listeria* Selective Supplement (BioRad, South Africa) according to ISO method 11290-1, 2017. A presumptive positive result for this test was recorded if blue/black coloured colonies were present on the red agar plates after 24 hours of incubation at 35°C. Since *L. monocytogenes* identification was based on the presence/absence of growth on the selective agar, no counts were recorded.

Statistical Analysis

Statistical analysis was performed using Microsoft Excel 2013 (Version 15.0.5249.1001) software. A 95% confidence interval was utilised to determine the significant differences (p <0.05). The calculations of means, standard deviations and bar graph construction was performed using Microsoft Excel 2013 coupled with XLStat (2020.1.3) (Addinsoft, 2020).

Collimated Beam Procedure

A collimated beam UV system was utilised for treatment of water samples at laboratory-scale. This device emits a single beam of UV light at a wavelength of 253.7 nm, which is deemed the germicidal wavelength of microorganisms as it targets and damages DNA (Bolton & Cotton, 2008). A simplified design can be seen in Fig. 1 below.

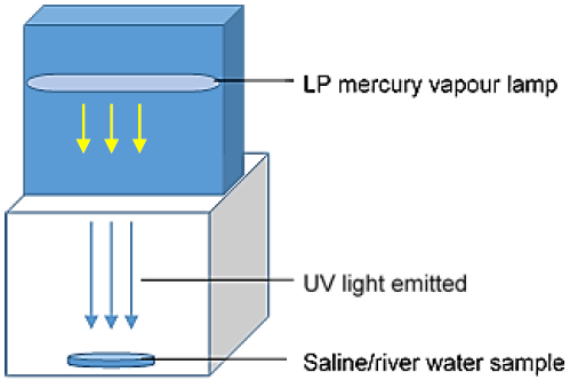


Figure 1 indicates the design of the bench-scale collimator device used to perform the laboratory-scale UV experiments (Berson, Netherlands) (Olivier, 2015)

This instrument utilizes an Amalgam LP mercury vapour lamp (UV-Technik, Germany) that has an output power of 40 W and an arc length of 25 cm. The intensity of the UV light is determined at the surface of the sample before each treatment is applied, and is measured using an ILT1400 radiometer (International Light Technologies, USA) which is coupled with a XRL140T254 detector (International Light Technologies, USA).

Research Design - *Establishment of a microbial and physico-chemical profile of the four rivers and investigating the effect of LP UV treatment on microbial population.*

The experimental design of this study was structured in order to meet the two previously stated objectives. Firstly, the physico-chemical analyses and microbial tests conducted before UV treatment were used to establish a microbial and physico-chemical profile for each river over a number of sampling occasions. Secondly, the microbial analyses before and after UV radiation provided an indication of the efficacy of this method for water disinfection, as well as to determine indicator and pathogen dose responses to UV radiation. The four selected rivers were sampled five times each over the summer irrigation period, from October 2019 – January 2020. Samples were analysed for physico-chemical parameters before UV treatment. Microbial analyses were conducted before and after three different UV doses; 20, 40 & 60 $mJ.cm^{-2}$. The dosages were applied by exposing the sample to three consecutive doses of 20 $mJ.cm^{-2}$, resulting in the final sample receiving a dosage of 60 $mJ.cm^{-2}$.

For each sample the UVT % of the river water was first established. The UV light intensity of the UV lamp was then measured at the surface of the sample and then calculations were performed to determine the exposure time required for each UV dose. The UV lamp was allowed to heat up for 10 minutes before the light intensity value was recorded. As the sample depth in the beaker decreases with each dose, the exposure time decreases too, and therefore, the calculation for exposure time needs to be performed at each sample depth. These sample depth changes were determined before each experiment with sterile water to minimise time taken between dose applications. The physico-chemical tests performed for each river water sample aid in the understanding of how the physical water characteristics may impact the disinfection efficacy.

The required volume for the microbial tests before UV treatment were aseptically removed, directly from the sampling bottle (Fig. 2), after which a dilution series was prepared (Fig. 3). A volume of 153 mL of river water was needed for all microbial tests, conducted after all three UV doses were applied. This volume of water was transferred to a sterile 500 mL beaker containing a magnetic stirrer. The magnetic stirrer was set on a medium speed, this selected speed was utilised for all tests that followed. After dose one was applied, the required amount of water was removed for the respective microbial tests, as indicated in Figures 2 & 3. The beaker was placed back into the collimated beam device, and the same procedure was repeated for doses two and three. No evaporation of water during the application of UV treatment was noted. The results were compared with the Irrigation Water Guidelines (DWAF, 1996a), where possible, to determine the suitability of this water for use, as well as to determine the efficacy of this treatment method. Microbial strains that survived UV radiation and pathogens identified from the river water before radiation, were isolated for future study.

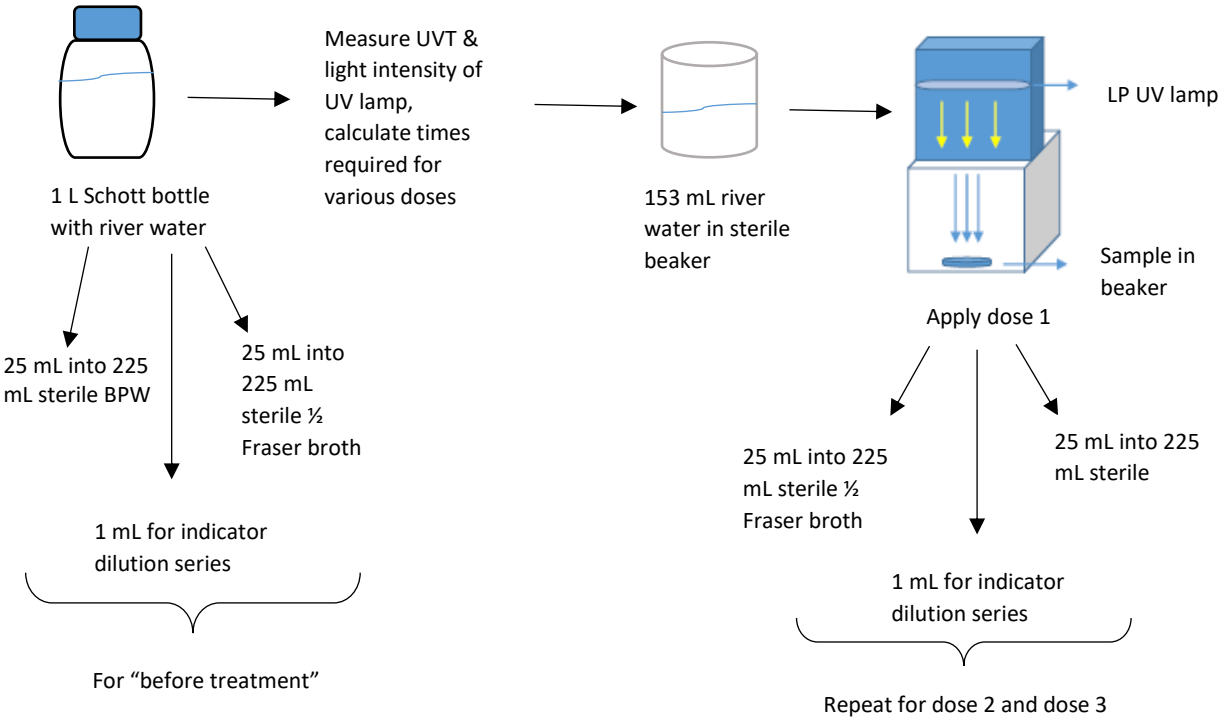


Figure 2 Schematic diagram of the post-sampling process, preparing samples for UV radiation, and the respective water sample quantities required for microbial tests

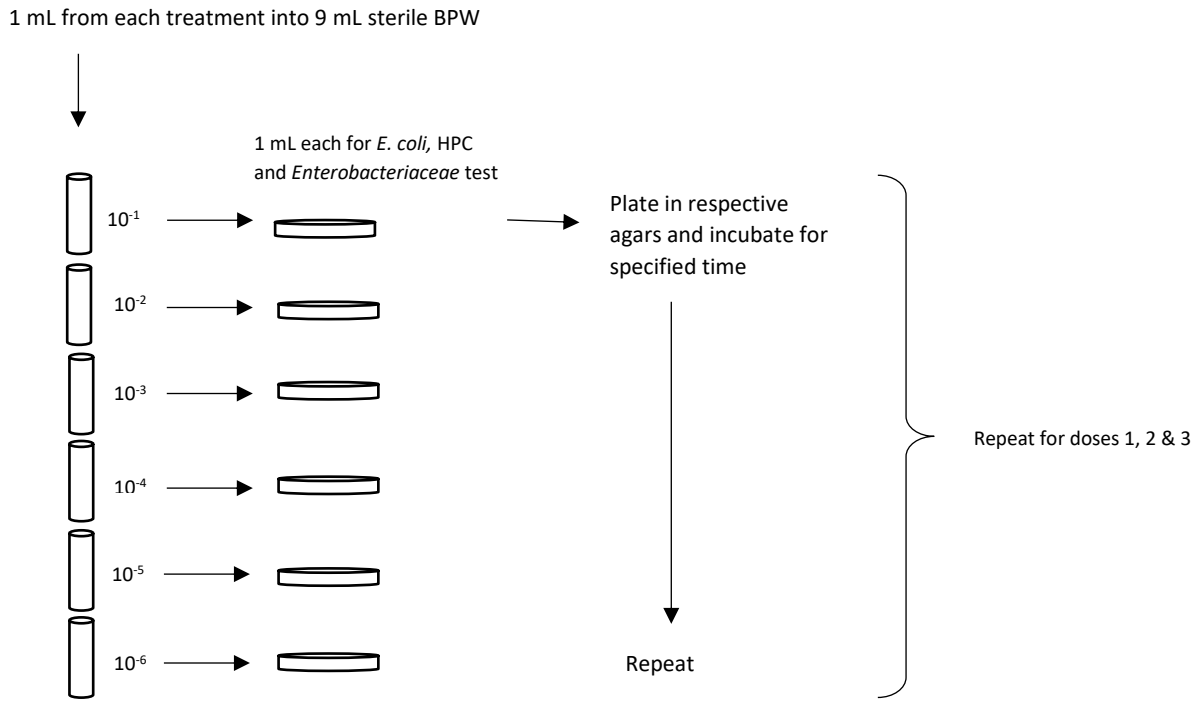


Figure 3 Schematic diagram of the dilution series process that was performed in this study before and after UV radiation, to determine the efficacy of disinfection of microorganisms present in river water with UV radiation at various doses

Isolation and storage of microbial strains for future testing

For the isolation and storage of isolates, the following procedure was followed. A single colony was removed from each of the agar plates, and streaked onto a Nutrient Agar plate and incubated at 37°C for 24 hours (Oxoid, South Africa). After incubation, a single colony was then removed from the agar plate using a sterile loop, and inoculated into 5 mL of sterile Tryptone Soya Broth (Oxoid, South Africa). This inoculum was incubated at 37°C for 24 hours, after which 800 µL of the TSB-bacterial suspension was then added to a sterile Cryovial, along with 800 µL of a sterile 50 % ($v.v^{-1}$) glycerol solution and gently mixed. This resulted in a bacterial stock solution with a final concentration of 25 % ($v.v^{-1}$) glycerol. Organisms isolated from this study were stored in glycerol at -80°C for further analysis, as described in Chapter 4.

Results and Discussion

***Objective 1**: Establishment of a microbial and physico-chemical profile of the four rivers*

In this part of the study, a microbial and physico-chemical profile was established for each of the four selected rivers by sampling each river five times. The physico-chemical results, which are indicators of physical and chemical water quality were included to determine the correlation between water quality and treatment efficacy. The microbial quality of the rivers are reflected in the results presented in Figures 4 and 5, as well as in Table 3.

The results of all physico-chemical tests are indicated in Table 2 for all four rivers over the five sampling occasions. The Irrigation Water Quality Guidelines (DWAF, 1996a) specify the requirement to test for the pH, TDS, TSS and electrical conductivity values of water samples only. The addition of tests such as the chemical oxygen demand (COD), alkalinity, UVT % and turbidity were included as these parameters, in conjunction with the previously stated water quality tests, provide a thorough indication of the water quality. These parameters play a major role in the efficacy of the treatment method for disinfection and should therefore, be taken into account when determining treatment application. Wherever possible, the Irrigation Water Quality Guidelines were consulted to determine whether the selected river sources were compliant in terms of water safety (DWAF, 1996a).

A total of 35% of the river water samples exceeded the guideline recommedation for alkalinity values. However, it can be noted that the alkalinity readings of all five sampling ocassions of the Mosselbank River dramatically exceeded the guideline limit of < 120 mg $CaCO_3.L^{-1}$. Spelman (2003) states that an alkalinity value above 80 mg $CaCO_3.L^{-1}$ allows for the most effective buffering capacity against environmental changes. The significantly higher alkalinity values (165 – 290 mg $CaCO_3.L^{-1}$) noted from the Mosselbank River could be due to the fact that this sampling location was situated downstream from a wastewater treatment plant. Baharvand & Danesvar (2019) states that elevated alkalinity values in wastewater from treatment plants are not uncommon. A study performed in a wastewater treatment plant in Iran noted that alkalinity values from raw sewage dropped from 380 - 390 mg $CaCO_3.L^{-1}$ to 300 - 350 mg $CaCO_3.L^{-1}$ after the first treatment process. Wastewater with high alkalinity values that is released into the surrounding rivers will cause increases in the alkalinity values of the river. Therefore, the impact of wastewater effluent on the water quality profile of rivers used for irrigation purposes should be considered.

Table 2 Physico-chemical characteristics of the four selected rivers used for irrigation over five sampling occasions during the summer irrigation period (October 2019 – January 2020)

	Plankenburg						Eerste						Mosselbank						Franschhoek					
Parameter	**1**	**2**	**3**	**4**	**5**	**Avg.** *SD*	**1**	**2**	**3**	**4**	**5**	**Avg.** *SD*	**1**	**2**	**3**	**4**	**5**	**Avg.** *SD*	**1**	**2**	**3**	**4**	**5**	**Avg.** *SD*
UVT %	60.3	60.0	64.5	61.2	31.2	55.4 *13.7*	62.2	62.0	63.9	56.1	52.1	59.3 *5.0*	22.2	30.0	28.3	25.0	30.2	27.1 *3.5*	80.0	80.0	79.2	80.2	49.1	73.7 *13.7*
TDS (mg. L^{-1})	180	160	143	165	485	226 *145*	245	316	285	297	272	283 *27*	831	850	802	903	540	786 *142*	96	95	96	83	244	123 *68*
EC ($mS.m^{-1}$)	0.30	0.19	0.17	0.22	0.55	0.28 *0.16*	0.34	0.43	0.32	0.37	0.34	0.36 *0.04*	1.0	1.16	0.91	0.99	0.63	0.93 *0.20*	0.13	0.11	0.14	0.13	0.26	0.15 *0.06*
COD (mg O_2. L^{-1})	29	22	19	52	202	63 *79*	25	12	18	34	16	21 *9*	59	50	57	47	47	52 *6*	12	10	10	13	24	14 *6*
Turbidity (NTU)	18.5	15.5	11.1	16.0	27	17.6 *5.9*	3.4	3.4	3.5	2.6	2.3	3.0 *0.6*	9.9	7.7	7.1	17.8	9.6	10.4 *4.3*	2.0	2.6	2.3	2.2	8.9	3.6 *3.0*
TSS ($mg.L^{-1}$)	19	19	6	5	18	14 *7*	9	11	8	4	14	9 *3*	9	24	8	13	22	15 *7*	6	5	1	3	7	4 *2*
pH	7.21	6.91	6.88	6.72	6.48		7.59	7.41	7.28	7.38	7.35		7.39	7.49	7.31	7.43	7.19		6.76	6.78	6.75	6.79	7.14	
Alkalinity (mg.L $CaCO_3^{-1}$)	75	90	63	70	168	93 *43*	106	106	93	100	95	100 *6*	290	205	220	270	165	230 *50*	50	45	55	45	135	66 *39*

SD – Standard deviation

The alkalinity values of the remaining three rivers fell within guideline recommendations (<120 mg $CaCO_3.L^{-1}$), except for the fifth sampling ocassion of the Plankenburg (168 mg $CaCO_3.L^{-1}$) and Franschhoek Rivers (135 mg $CaCO_3.L^{-1}$) (Table 2). The river water levels in the Franschhoek River on this sampling ocassion appeared to be significantly lower with a slower flow rate, and was coupled with increases in all other physico-chemical characteristics. Most notably, a drop in UVT % from 80% to 50%, a quadrupled turbidity value (8.9 NTU) and double the COD value (24 mg $O_2.L^{-1}$) – when comparing this sampling occasion to the previous four sampling ocassions (Table 2). Similar findings were noted for the Plankenburg River on the fifth sampling ocassion.

Water that contains high alkalinity values, and therefore, high levels of carbonates and bicarbonate ions, results in insoluble minerals formed by calcium and magnesium ions that remain in the soil after irrigation (Bauder *et al.*, 2014). Scale build-up from carbonates in irrigation water on drip or spray irrigation systems causes reduced water flow rates, and can therefore, negatively impact irrigation efficacy (Bauder *et al.,* 2014). Alkalinity is affected by dissolved salts and the oxidation of organic matter which wouldn't necessarily affect pH. It is possible to have a water sample that has a lower pH but still ranks high in alkalinity, therefore, still has the capacity to buffer changes in acidity levels, while being acidic (USEPA, 2011). There is a positive correlation between the TDS and the alkalinity of the water samples (Table 2). These findings agree with a study performed by Shroff *et al.* (2015) where a 0.88 correlation coefficient was reported between the TDS and alkalinity of water samples obtained from the environment. High levels of carbonates in water samples have been associated with the deposition or scaling on the UV lamp sleeve. This has an impact on the intensity of the UV radiation that reaches the water (USEPA, 2003). Consistent cleaning of the lamp sleeve is recommended for waters with high alkalinity values (USEPA, 2003).

The pH value of a water sample, according to Abdelrahim *et al.* (2013), has an effect on the biological and chemical reactions that occur in water, as well as having an impact on the solubility of metal ions. The specified pH range (DWAF, 1996a) for the irrigation water samples, 6.5 – 8.4, is relatively wide and it is noted that all river water samples fell within this range (Table 2). A general trend can be noted, where high alkalinity readings are associated with higher pH values (Table 2). This correlates with a statement made by Valdez-Aguilar *et al.* (2009), where high pH values in irrigation water are associated with high concentrations of carbonates and bicarbonates. The microbial activity of water samples is directly impacted by the water pH (Jin & Kirk, 2018). This is due to the fact that the pH is responsible for influencing the acitivites and growth of the microbial communities.

Turbidity is determined by the amount of light that is scattered by particles within a water sample, but this does not include any settled solids (Perlman, 2014). The guideline limits

(DWAF, 1996a) do not include a specification for the allowed turbidity for irrigation water. The Water Quality Guidelines for Domestic Use (DWAF, 1996c) states that for turbidity values exceeding 10 NTU, the water carries an associated risk of disease, and this guideline was used in this study as a reference basis to comment on the water quality of the four selected rivers. Of the four rivers studied, the Plankenburg River consistently had higher turbidity values (11.1 – 26.9 NTU), all of which exceeded the guideline limit. The other rivers all had values below 10 NTU (except on one occasion for the Mosselbank River) (Table 2). Interestingly, the Plankenburg River also had the most consistent presence of pathogenic microorganisms of all the rivers investigated (Table 3). Rapid clogging of irrigation equipment can be attributed to the TSS and turbidity of a water sample and needs to be taken into account to ensure that the UV treatment is applied consistently to all water samples (Ribeiro *et al.,* 2004). A study performed by Ribeiro *et al.* (2004) noted a positive relationship between turbidity and TSS readings in irrigation water in Brazil. Furthermore, high-coefficient correlations were noted between the TSS and bacterial and algae concentrations in irrigation water. Similar findings were noted for increased turbidity levels (Ribeiro *et al.,* 2004).

The total solids are the sum of the settleable solids, the suspended solids (TSS) and the dissolved solids (TDS) (USEPA, 1999). The TSS of a water sample includes sand, silt, clay, mineral precipitates as well as biological matter, according to Butler & Ford (2018), which includes all particles that would be held back by a 2 µm filter (USEPA, 1999). The Mosselbank River had consistently high dissolved solids content (540 – 903 $mg.L^{-1}$) compared to the other three rivers (average 82 – 485 $mg.L^{-1}$) (Table 2). This was in contrast with the TSS values of the Mosselbank River (8.2 – 24.2 $mg.L^{-1}$), that were approximately in the same range as those observed for the other three rivers over the sampling occasions (1.3 – 19.4 $mg.L^{-1}$) (Table 2). This is an indication of a higher concentration of dissolved salts and minerals, which could provide an explanation for the higher alkalinity readings in the Mosselbank River. This emphasises the potential impact that effluent from the wastewater treatment plant, situated upstream from the sampling location, may have on the river water profile.

A linear relationship between the turbidity and TSS has been proposed by Hannouche *et al.* (2011). High turbidity, which is a reflection of high levels of TDS and TSS in the sample, implies a high level of organic and inorganic pollution (Johnson *et al.,* 2010). The relationship between TDS and TSS has been reported as not constant or proportional over time (Butler & Ford, 2018). The variability in these two factors is, therefore, too great to allow for a direct relationship to be established. Higher TDS readings are noted in the Eerste River (average of 283 $mg.L^{-1}$) compared to the Plankenburg River (average of 226 $mg.L^{-1}$), while the opposite is reported for the TSS readings (averages of 9 and 14 $mg.L^{-1}$ for these two rivers, respectively) (Table 2). This could mean that the higher concentration of dissolved solids in

the Eerste River does not have a considerable influence on the UVT % values (which remained relatively similar across the five sampling occasions of these two rivers). This could indicate that the suspended solids may play a greater role in the scattering of light for the Plankenburg River, or that the organic matter is resulting in the scattering of light.

A report by Islam *et al.* (2017) states that the TDS of a water sample correlates positively with the electrical conductivity (EC) and directly affects the pH. As the TDS value increases, the electrical conductivity increases, too, and a more acidic pH is reported (Islam *et al.,* 2017). Bauder *et al.* (2014) states that the electrical conductivity of irrigation water is the most influential water quality parameter with regard to crop productivity. This is due to the fact that, with higher EC values in irrigation water, the less water is available for the plant as it is unable to compete with the ions present in the soil solution for water (Bauder *et al.,* 2014). Therefore, water available for transpiration of the plant decreases as the EC increases which ultimately leads to reduced yield potential. In this study, the high alkalinity values observed for the Mosselbank River generally correlated with slightly higher pH values (Table 2). The stated correlation between an increased EC and TDS values hold true across all sampling occasions for all four rivers (Table 2). Rusydi (2018) states that the relationship between electrical conductivity and total dissolved solids in water sources as one that correlates linearly (R^2 = 0.97). This can be calculated as:

TDS = 0.65 x EC (where EC is $\mu S.m^{-1}$ and TDS is $mg.L^{-1}$)

When applying this calculation to the recorded values for EC and TDS (Table 2), it is evident that the correlation is correct. All rivers have electrical conductivity values within the specified limit (DWAF, 1996a). Lowered EC readings, according to Edokpayi *et al.* (2018), are most commonly reported in the rainy seasons of an area, which is as a result of the dilution effect from increased precipitation. Increased evaporation during the drier season can lead to increased levels of dissolved ions in river water (Edokpayi *et al.,* 2018). As the current study was performed in the drier season only, with limited rainfall, this correlation could not be observed.

Chemical Oxygen Demand (COD) is a measure of the total amount of oxygen required to oxidise all organic material into carbon dioxide and water (USEPA, 1999). There is currently no stipulation within the irrigation guidelines (DWAF, 1996a) in South Africa regarding the COD of a water sample for either irrigation water or domestically-used water. Therefore, the Guidelines for Industrial Use (DWAF, 1996b) were consulted instead. These guidelines state that acceptable limits for COD in irrigation water is < 75 $mgO_2.L^{-1}$ as irrigation water falls within Category 4 Utility Water of Industrial Processes, which allows for the discharge of an effluent, without the clogging of equipment.

Relatively consistent values for COD content was reported for each river over the sampling occasions, where the limit was exceeded on one occasion only in the Plankenburg River (Table 2). The Mosselbank River consistently had the highest COD values, with an average of 52 mg $O_2.L^{-1}$ over the five sampling occasions. The Franschhoek River consistently had the lowest average COD values, where an average reading of 14 mg $O_2.L^{-1}$ was reported (Table 2). Sampling occasion five for the Plankenburg River being the exception, where a major increase in COD values was recorded (202 mg $O_2.L^{-1}$ respectively), compared with previous values (Table 2). It was noted that there was a slower flow rate, and appeared to have much lower water levels than the previous sampling occasions of this river. It was also noted that there was rainfall in the week before the fifth sampling occasions of the Eerste and Mosselbank Rivers and not before this sampling occasion of the Plankenburg and Franschhoek Rivers. A study performed by Momou *et al.* (2017) noted that the suspended particulate matter, which includes the biological oxygen demand and COD was four to ten times higher following a period of rainfall in surface waters. Contrastingly, a report by Mosley *et al.* (2015) states that periods with limited rainfall have been associated with increases in turbidity as well as dissolved oxygen concentrations as a result of reduced dilution from rainfall. This finding could be used to explain the increases in TDS, COD and turbidity readings from the Plankenburg and Franschhoek Rivers. The increase in COD readings, observed in all rivers, correlated with much lower UVT % readings, compared to values recorded during previous sampling occasions of these rivers.

The large increases in TDS and turbidity values in the Plankenburg and Franschhoek Rivers on the last sampling occasion (Table 2), coupled with the major reduction in UVT % indicates the interlinking relationship between these factors. Wu *et al.* (2011) states that due to the fact that COD is predominantly an indicator of the organic pollution level in the water sample, and many of these pollutants absorb radiation in the UV region, it can significantly impact the UVT % in water, and therefore, ultimately influence UV treatment efficacy.

When observing the overall physico-chemical parameters of the four selected rivers, the Mosselbank River consistently had the lowest water quality (Table 2). This was coupled with the lowest UVT % (with a range of 22-30%) and can be attributed to the high TDS values recorded. It is evident that the TDS of the water samples play a role in affecting the UVT %, due to the fact that notable decreases in UVT % are consistently correlated with increases in TDS. Therefore, the TDS may be impacting the UVT % more significantly than was expected. The impact of low UVT % on disinfection efficacy has been reported by Olivier (2015), and this needs to be taken into account when determining pre-treatment steps as well as the dosage application. This river will be considered the worst-case scenario in terms of physical characteristics. The Franschhoek River was consistently the river with the best water quality,

which was coupled with the lowest indicator counts (Fig. 6A) as well as pathogen presence (Table 3). The UVT % of this river fell within the range of 49-80% (Table 2). This river will be regarded as the best-case scenario for the rest of the study. The Eerste and Plankenburg Rivers had UVT % readings of between 52-62%, with the exception of the last sampling event of the Plankenburg River. These UVT % readings can be considered as adequate to ensure effective disinfection of the water.

The UVT % of water samples is known to be greatly impacted by the turbidity as well as the TSS (Farrell *et al.,* 2018). This is due to the fact that, much like suspended solids, microorganism aggregates can be enclosed within the particulates in the turbid samples, which increases the resistance of microorganisms to UV penetration dramatically (Farrell *et al.*, 2018). Turbidity is seen to be a factor that most greatly influences the efficacy of UV treatment of water, where an inverse relationship between UVT % and turbidity has been reported (Gurol, 2005). The findings in the current study correlate with this statement, where rivers that had the lowest turbidity readings consistently had the highest UVT %. Jones *et al.* (2014) states that the relationship between turbidity and UV treatment efficiency is, however, inconsistent. This is because factors that contribute to an increase in turbidity show great variability in the ability to block or absorb UV radiation. Clarke and Bettin (2006) state that a link between low turbidity values and the absence of pathogenic microorganism cannot be guaranteed. Although there are seemingly conflicting opinions regarding the impact of turbidity on UV treatment efficacy, it is evident that as the turbidity value increases, the exposure time required to deliver a specific dose increases as the UVT % is decreased. Therefore, from an economic standpoint, the turbidity does indeed play a major role in this method of water disinfection. It is important to implement a pre-treatment step to ensure that the UV treatment is applied to a consistent standard of water. Olivier (2015) states that inorganic as well as organic compounds are able to absorb UV light in water samples, and therefore, the COD and TDS are inversely proportional to the UVT %.

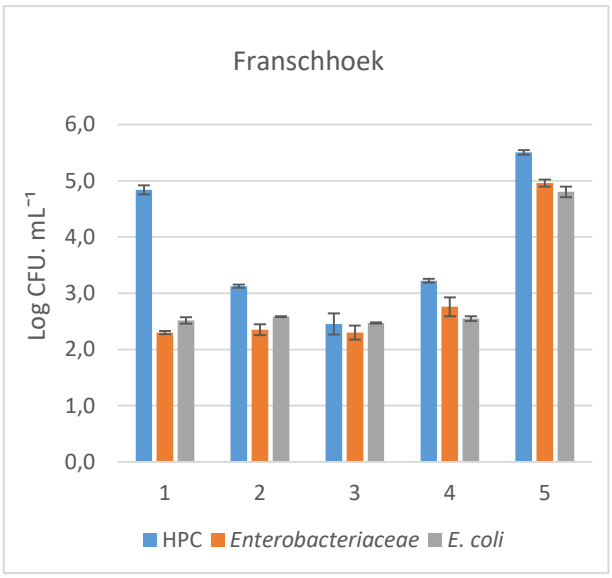
The factors that are responsible for impacting the efficacy of UV treatment for water disinfection are the turbidity, suspended solids, dissolved solids, COD and UVT % of a water sample (Hassen *et al.,* 2000 & Gayán *et al.,* 2014). Suspended solids, according to Abdul-Halim & Davey (2016), reduce the efficacy of this treatment method either by shielding the microorganisms from UV radiation or by absorbing the UV radiation, thereby, reducing the energy available for microbial inactivation. When UV light is absorbed by suspended solids, it is no longer available for inactivation of microorganisms and should, therefore, be taken into account when determining the dosages for UV treatment (Liu, 2005). In order to achieve complete disinfection of water samples, it is evident that the physico-chemical parameters play

a major role in impacting treatment, and should be consistently monitored to ensure an effective treatment.

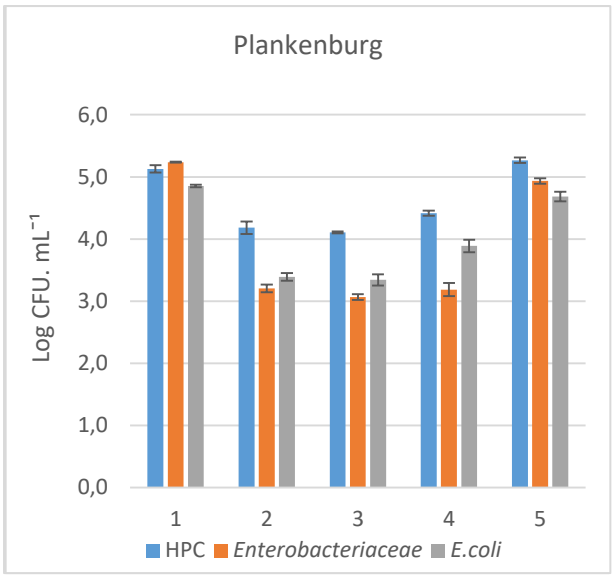
The observation of the microbial and physico-chemical characteristics of four rivers over the summer irrigation period (October 2019 – January 2020), provides information about the water profiles of these different river sources with differing upstream activities and overall water quality. This, therefore, provides information about varied river water quality and allows for broader UV treatment parameter optimisation that accommodates for the worst-case scenario.

In this study, microbial indicator levels present in the four selected rivers over the five sampling occasions were determined and are represented in Figs. 4 A-D as log cfu.mL^{-1}. Indicator organism tests included HPC, *Enterobacteriaceae* and *E. coli.* The error bars shown in the following figures represent standard deviation, which indicate the variation between duplicate plates poured, across six decimal dilutions.

As mentioned, the rivers were sampled five times each over the summer irrigation period (October 2019 – January 2020). The microbial indicator counts were determined using a dilution series of 10^{-1} – 10^{-6} and standard plating methods. Dilutions were plated in duplicate, and the results were averaged. The results were recorded as log cfu.mL^{-1} (Figs. 4 A – D, 5, 6 A – D, 7 A – D and 8 A – D). The pathogen tests were performed on a presence/absence basis only. The Irrigation Water Guidelines (DWAF, 1996a) states only that the *E. coli* counts of an irrigation water sample should fall within 1 000 cfu.100 mL^{-1} to be deemed safe. This correlates to 10 cfu.mL^{-1} or log 1 cfu.mL^{-1}. There is no requirement to test for pathogens such as *Salmonella* spp. and *L. monocytogenes* in the current guidelines. At present, few studies exist regarding the prevalence of these pathogens in South African surface waters in South Africa, and knowledge gaps exist on how effectively UV disinfection can be used to reduce pathogen levels present in surface waters worldwide.



4A



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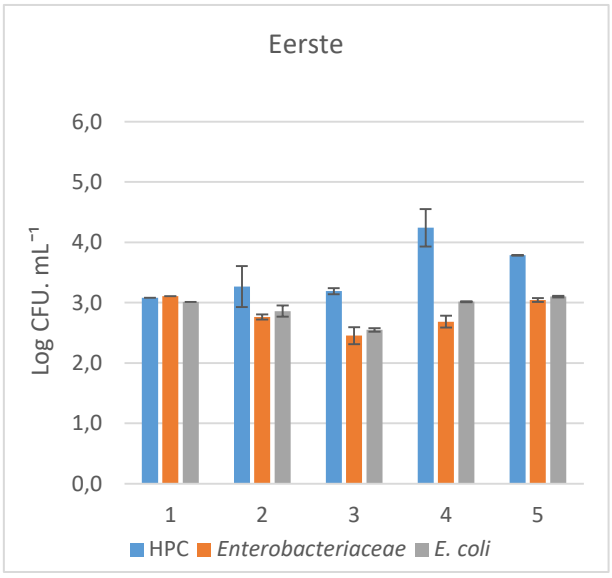

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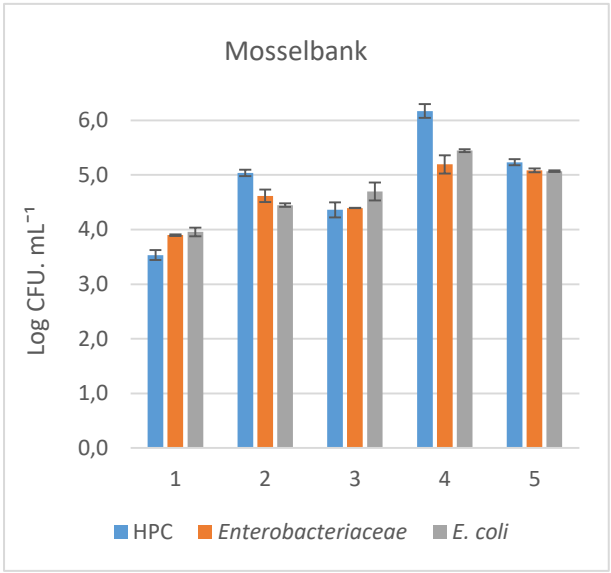


4D

Figure 4 A-D Results of indicator organism colony counts before UV treatment is applied, expressed as log cfu.mL^{-1}, with standard deviation error bars included

Figures 4 A – D provide an indication of the great variability in water quality, not only between the four selected rivers but also between the sampling occasions for a river over the summer irrigation period. These colony counts were, where possible, compared to the Irrigation Water Guidelines (DWAF, 1996a) to determine the fitness for use. The fourth sampling occasion of the Mosselbank River saw an *E. coli* count of log 5.45 ± 0.03 cfu.mL^{-1} which equates to 279 000 cfu.mL^{-1}. This was the highest *E. coli* count recorded of all four rivers. The lowest recorded *E. coli* count was sampling occasion three in the Eerste River. A log 2.55 ± 0.01 cfu.mL^{-1} equates to an average of 355 cfu.mL^{-1}. Even the lowest *E. coli* colony

count observed dramatically exceeded the guideline limit. This confirms the need for effective water disinfection treatment prior to irrigation. It can be noted in Figures 4 A – D that there are some instances where the *E. coli* counts are higher than the *Enterobacteriaceae* counts. This could primarily be as a result of the methodology, specifically, the different agar used that caused this result.

Figure 5 provides a visual representation of the average recorded values over the five sampling events per river for each indicator population. This provides an overall microbial quality indication of each of the rivers' for the entire summer irrigation period. Increased counts did, in some instances, correlate with worsening physico-chemical characteristics. For instance, a significant increase in indicator colony counts was noted from the first four sampling occasions of the Franschhoek River to the fifth sampling occasion (Fig 4A). This correlated to a substantial UVT % decrease, and all other physico-chemical results (except pH) almost doubled, compared to the previous four sampling occasions (Table 2). Likewise, a similar trend was observed between sampling occasions four and five of the Plankenburg River (Table 2). It can therefore be noted that, a tentative correlation could be made between the physico-chemical and microbial quality characteristics of the water sampled. The Plankenburg and Mosselbank Rivers were consistently the most contaminated rivers, considering the microbial indicator counts (Fig. 4B & 4D). Even though the Eerste and Franschhoek Rivers (Fig. 4C & 4A) had *E. coli* counts that were lower than the other two rivers, these counts still markedly exceeded the water quality guideline limits. Therefore, on the basis of *E. coli* levels only, all four rivers were deemed unacceptable for agricultural use without pre-treatment.

Escherichia is one of many genera that fall within the *Enterobacteriaceae* family. It can be noted from Figures 4 A–D that little variation existed between *Enterobacteriaceae* and *E. coli* counts. Statistical analysis was performed to determine if there are significant differences between the *E. coli* and *Enterobacteriaceae* counts observed across the five sampling occasions per river. The p-values obtained in this experiment were; 0.353, 0.608, 0.811, and 0.628 for the Plankenburg, Eerste, Franschhoek and Mosselbank Rivers, respectively. At a 95.0% confidence interval, no significant difference ($p<0.05$) was observed between the colony counts of *Enterobacteriaceae* and *E. coli.* A tentative conclusion could be drawn that the *Enterobacteriaceae* population predominantly consisted of *E. coli* strains. The HPC values vary greatly between rivers and across sampling occasions. No correlation can be made between HPC values and *Enterobacteriaceae*/ *E. coli* counts. Due to the fact that the HPC test is a non-selective test for the total aerobic bacteria in the water sample, it is difficult to draw conclusions as to what kind of contamination had occurred before certain sampling occasions where HPC counts were high, for example, sampling occasion one of the

Franschhoek River (Fig. 4A). Overall, these results provide a good baseline microbial profile for each river, which aids in the understanding of how best to disinfect the river water. Upstream activities, be it recreational or industrial, dumping of household waste and animal defecation may all play a role in the microbial populations in the river waters. Changes in weather, such as extreme winds, heat or rainfall may too play a role in these findings. Understanding how these factors fluctuate across the irrigation season in the Western Cape are of great importance to ensure that the most effective treatment is applied, taking the worst-case scenario into consideration.

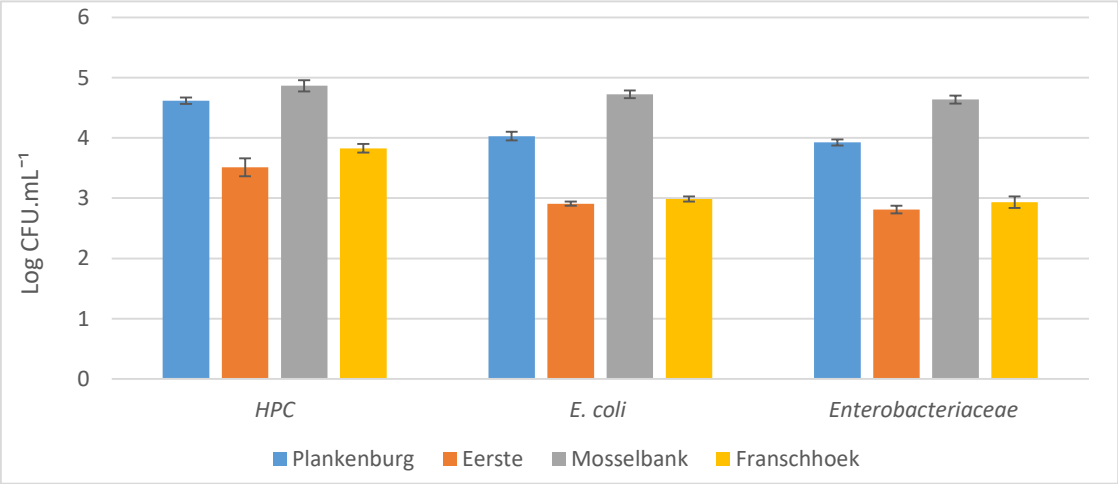


Figure 5 Average colony counts for all indicator organisms tested across five sampling occasions, indicated as log cfu.mL^{-1} with standard deviation bars included for error across all five sampling occasions per river

A report by Sigge *et al.* (2016) has highlighted the possibility of a potential food safety risk that is associated with the presence of pathogens in irrigation water. Sigge *et al.* (2016) proposed the importance of setting limits for important food pathogens in addition to *E. coli,* such as *L. monocytogenes* and *Salmonella* spp., in irrigation water, to ensure a reduction in foodborne outbreaks associated with fresh produce. The results of the pathogen tests done in this study are presented in Table 3, where the presence and absence of the pathogens *Salmonella* spp. and *L. monocytogenes* in the untreated river water samples are indicated.

The Plankenburg and Mosselbank Rivers, again, had the most consistent pathogen presence over the five sampling occasions. The Franschhoek and Eerste Rivers were the only rivers to have *Salmonella* spp. present on one sampling occasion only, which was sampling occasion five. It must be noted that there were extremely high temperatures in the week before testing, which may have influenced the presence of this pathogen. *L. monocytogenes* was present in all but one sampling occasion of the Eerste River. The Franschhoek River had the lowest prevalence of *Salmonella* spp. and *L. monocytogenes.*

Vasquez-Boland *et al.* (2001) states that *Listeria* spp. are saprophytic organisms, that feed on dead organic matter in the environment and are ubiquitous in nature. The presence of *Listeria* spp. in general in river water is thus, not surprising. Unfortunately, *L. monocytogenes* in particular carry pathogenic genes that can be activated the moment they enter the human body (Vasquez-Boland *et al*., 2001). The need to control this organism in an industry that involves minimally processed foods is therefore, of great importance to ensure consumer safety.

Table 3 Presence and absence of *Salmonella* spp. and *L. monocytogenes* in river water samples, prior to the application of UV radiation. Shaded blocks with a "+" sign represent a positive test for the specific organism over the five sampling occasions

River	Pathogen tested	1	2	3	4	5
Plankenburg	*Salmonella* spp.	+	+	-	+	+
	L. monocytogenes	+	+	+	+	+
Eerste	*Salmonella* spp.	-	-	-	-	+
	L. monocytogenes	+	+	+	+	-
Mosselbank	*Salmonella* spp.	-	+	-	+	+
	L. monocytogenes	+	+	+	+	+
Franschhoek	*Salmonella* spp.	-	-	-	-	+
	L. monocytogenes	-	+	+	-	+

The consistent presence of *Salmonella* spp. and *L. monocytogenes* in the four rivers across the five sampling occasions indicate that these are not irregular findings. This indicates that these rivers are consistently contaminated and therefore, pose a great risk for farmers and other individuals using the water without pre-treatment. McEgan *et al.* (2013) states that the prevalence of indicator organisms such as *E. coli* in surface waters can be an indication of the presence of pathogenic microorganisms, such as the ones tested here. This finding correlates with the colony counts observed in the Mosselbank and Plankenburg Rivers, where high indicator counts are correlated with a consistent pathogen presence. These findings further exacerbate the need for guidelines in South Africa to include pathogen limits, to ensure that these microorganisms can be treated effectively and will not pose a risk for consumers of fresh produce irrigated with these waters.

***Objective 2**: The efficacy of low-pressure UV radiation on microbial populations in river water*

The effect of UV radiation on microbial counts was evaluated using a laboratory-scale collimated beam LP UV system. These water samples were exposed to three UV doses, and the microbial load after each applied dose was tested and recorded (Figs. 6 – 8 & Tables 3 and 5). In this study, it was observed that UV radiation is an effective treatment method, as all microbial counts were reduced to a level that would be considered adequate for use, according to the Irrigation Water Guidelines (DWAF, 1996a).

Table 4 Exposure times (min) calculated from the UVT % of each river water sample, UV lamp intensity (78.8 mW.cm^{-2}) and sample depth for the three specified UV dosages (mJ.cm^{-2}), based on data obtained in the first sampling occasion

Rivers	Plankenburg River	Eerste River	Mosselbank River	Franschhoek River
	UVT %			
	60.3	62.2	22.2	80
UV doses (mJ.cm^{-2})	Exposure time (min: sec)			
20	9:02	8:41	22:24	6:06
40	16:29	15:55	38:42	11:36
60	22:31	21:50	49:22	16:35

Table 4 provides an indication of the exposure times required for UV radiation at three different doses (20, 40, 60 mJ.cm^{-2}), using a lamp intensity reading of 78.8 mW.cm^{-2} and the respective UVT % reading of each river. Kahn *et al.* (2015) states that scattering of UV light in highly turbid water results in the reduction of UVT %, and this leads to extended exposure times for effective disinfection. In Table 4, water from the Mosselbank River had a UVT % of 22.2% (on sampling occasion 1), which required an in exposure time of 22:24 (min: sec) to ensure that a dose of 20 mJ.cm^{-2} was applied. In contrast, water from the Franschhoek River required an exposure time of 6:06 to achieve the same dose, based on the UVT % of 80% (Table 4). The time taken to apply the second dose (40 mJ.cm^{-2}) and third dose (60 mJ.cm^{-2}), as can be seen in Table 4 decreases with each dose, this is due to the fact that the respective amount of sample is removed for microbial testing after each dose. The sample depth, therefore, decreases and reduces the time required to apply the next dose as the calculation for exposure times includes the sample depth. It is evident that, to apply a 60 mJ.cm^{-2} dose, a significant amount of time is required, which impacts efficiency and economic feasibility. Therefore, implementing pre-treatment steps to improve UVT %, such as filtration, the

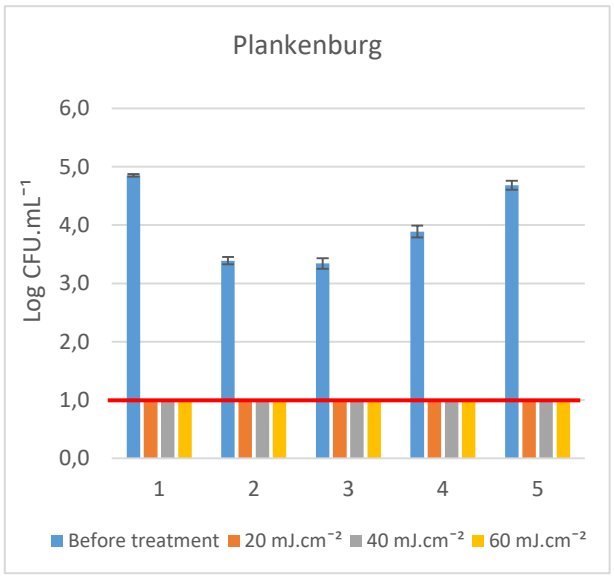
exposure time might be dramatically reduced and increase throughput greatly. Sivhute (2019) states that by utilising a pre-treatment step to ensure the physical water quality is improved, could translate to increased log reductions once UV radiation is applied. The microbial disinfection efficacy and process efficiency are factors of great importance in any water treatment method. Ensuring that the greatest disinfection occurs in an economically feasible timeframe is of utmost importance. When upscaling to a pilot-scale system, a medium-pressure (MP) lamp will deliver higher UV light intensities than the LP lamp utilised in the collimated-beam system, and would decrease exposure time significantly.

Okpara *et al.* (2011) states that chlorine, in sufficient doses, is able to disinfect water samples of microorganisms within 30 minutes. When one considers the negative environmental impact of chlorine as a disinfection method, it is also of great importance to take the economics into consideration. No matter the sample size, disinfection with chlorine takes a predetermined amount of time. So, an exposure time of 22 minutes (required for the Mosselbank River) (Table 4), as the worst-case scenario, is not nearly as long as the exposure time required for chlorine disinfection at any scale. Ultraviolet light intensities can be increased exponentially, especially through the use of MP UV systems, and therefore, the contact time can be significantly reduced. Another factor is that the cost of UV radiation does not increase linearly as it does with chlorine. Cost and time required for disinfection are two factors that, from a farmer's perspective, may govern the choice of which treatment method to use.

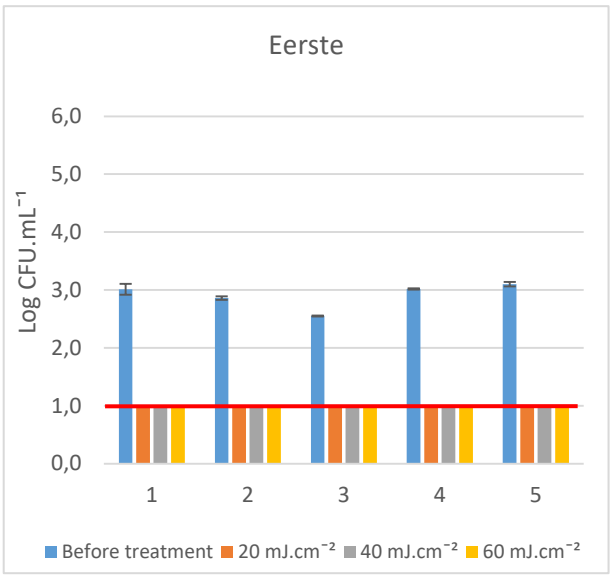
Results of indicator organism response to UV radiation

Water samples were exposed to UV light in a collimated-beam UV machine at laboratory-scale. The colony counts before treatment were recorded, along with the counts after exposure to the three selected UV doses. These results are shown in Figs. 6A-D – 8A-D and are expressed as log cfu.mL^{-1}. Table 5 provides an indication of the presence of the tested pathogens, before and after UV treatment. These results are expressed on a presence/absence basis.

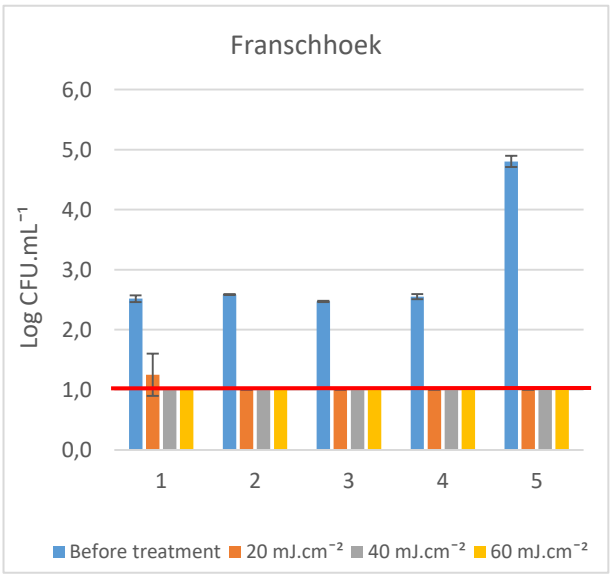
Escherichia coli



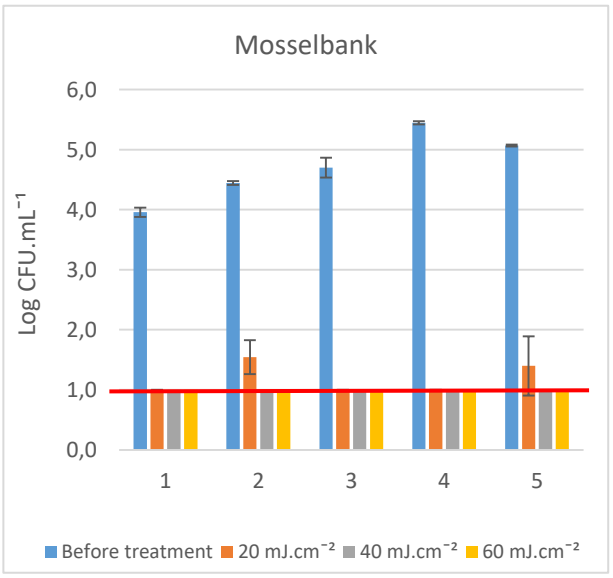
6A



6B



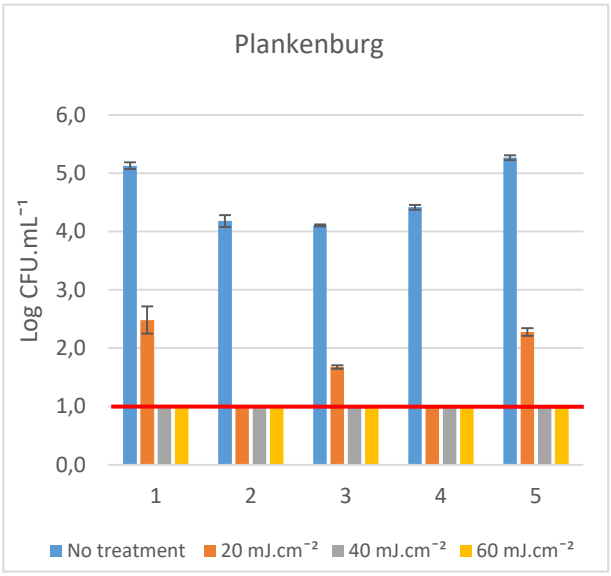
6C



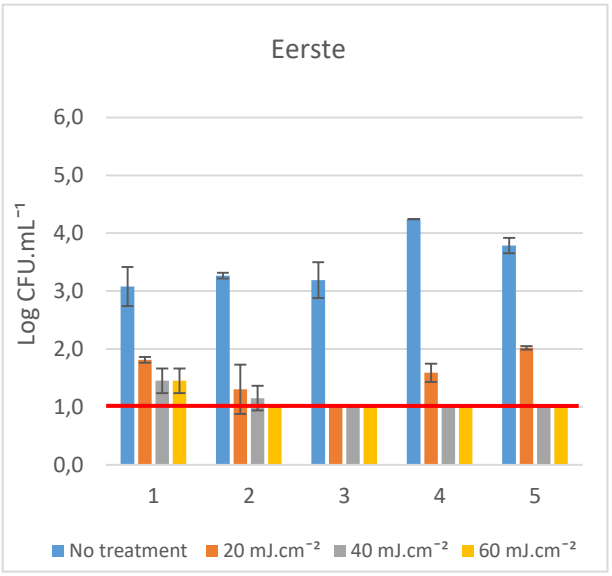
6D

Figure 6 A – D *E. coli* counts expressed as log cfu.mL^{-1} before and after three UV radiation doses for four rivers over five sampling occasions, where 1.0 log cfu.mL^{-1} is the detection limit, the red line at 1.0 log cfu.mL^{-1} indicates the lowest detection limit, below which there was no growth noted

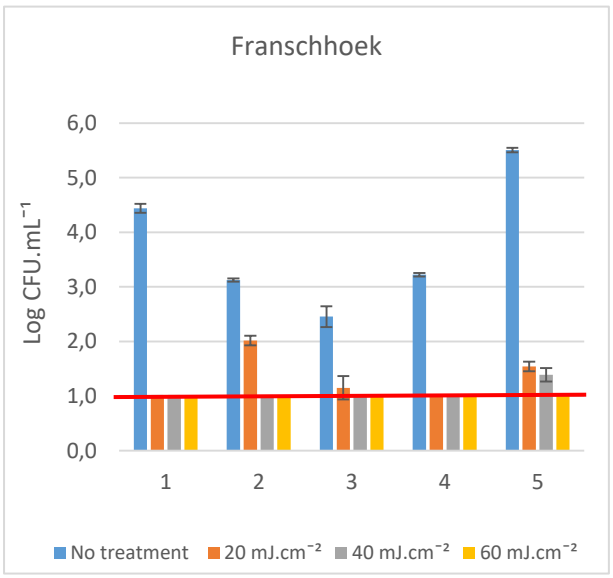
Heterotrophic Plate Count



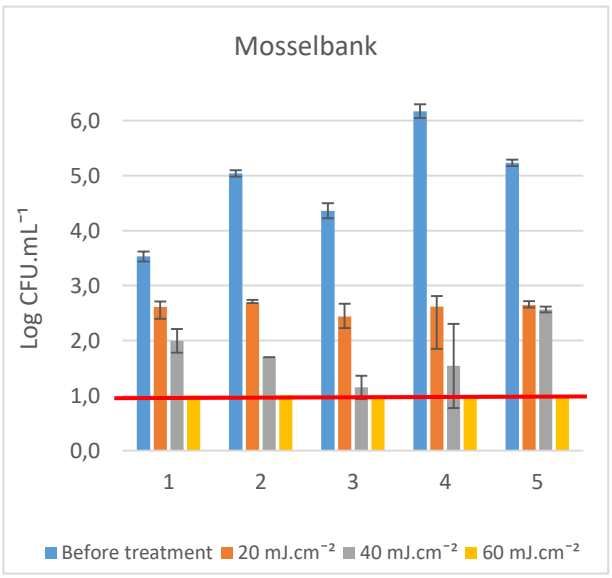
7A



7B



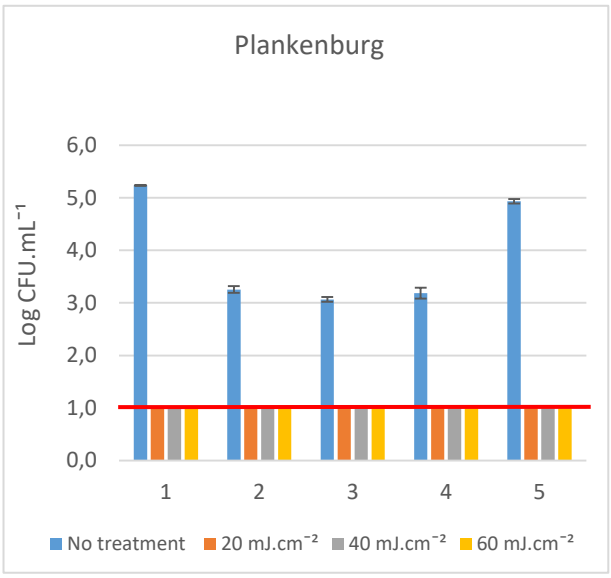
7C



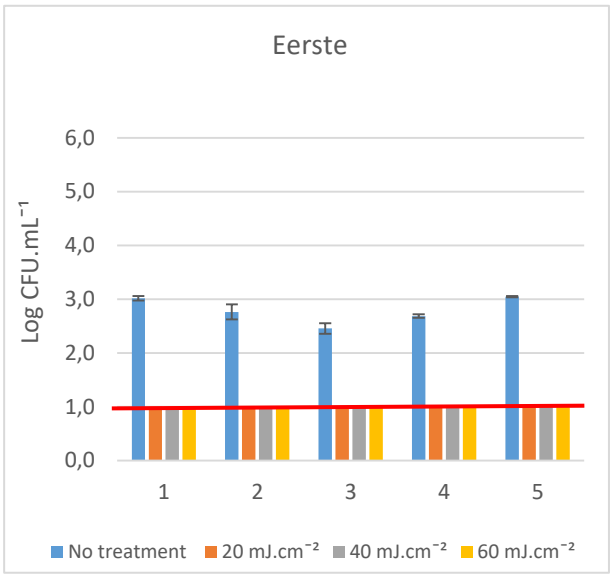
7D

Figure 7 A – D Heterotrophic Plate Count colony counts expressed as log cfu.mL^{-1} before and after three UV radiation doses for four rivers over five sampling occasions, where 1.0 log cfu.mL^{-1} is the detection limit, the red line at 1.0 log cfu.mL^{-1} indicates the lowest detection limit, below which there was no growth noted

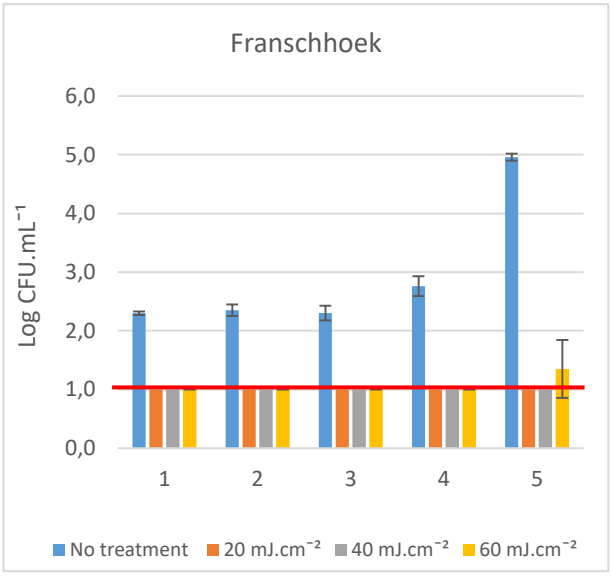
Enterobacteriaceae



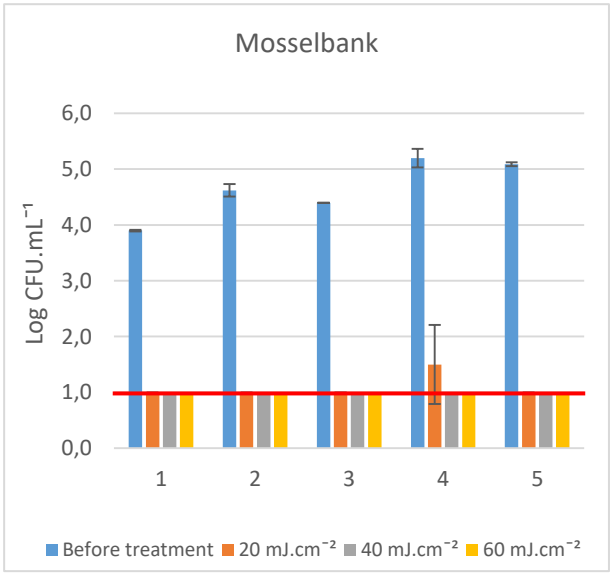
8A



8B



8C



8D

Figure 8 A – D *Enterobacteriaceae* colony counts expressed as log cfu.mL^{-1} before and after three UV radiation doses for four rivers over five sampling occasions, where 1.0 log cfu.mL^{-1} is the detection limit, the red line at 1.0 log cfu.mL^{-1} indicates the lowest detection limit, below which there was no growth noted

The log reductions recorded for all indicator organism tests, after the lowest dose of UV radiation applied (20 mJ.cm^{-2}), indicate the efficacy of this treatment method for disinfection. After 20 mJ.cm^{-2} of radiation is applied, the water quality, would be deemed safe for use according to the Irrigation Water Guidelines (DWAF, 1996a), for *E. coli* counts. Sivhute (2019) states that the sensitivity of a microorganism to UV radiation can be described by the degree of log reduction that is observed following radiation. Higher log reductions would be noted for UV sensitive organisms, than observed for UV resistant strains. The higher the initial microbial load, the larger the log reduction value will be. Log reduction values do not provide an indication of the initial microbial loads.

In this study, initial *E. coli* counts over the five sampling occasions, ranged between 7 900 – 291 000, 300 – 73 000, 350 – 1 260 and 1 870 – 74 000 cfu.mL^{-1}, for the Mosselbank, Franschhoek, Eerste and Plankenburg Rivers, respectively (Figs. 6A-D). The microbial reductions after UV radiation are presented in these figures, where no detectable colonies were present after the first dose (20 mJ.cm^{-2}) was applied, except for two sampling occasions of the Mosselbank River and one of the Franschhoek River (Figs. 6C & 6D, respectively). The HPC microbial levels ranged between 2 900 – 1 780 000, 200 – 340 000, 600 – 17 500 and 12 400 – 198 000 cfu.mL^{-1}, for the Mosselbank, Franschhoek, Eerste and Plankenburg Rivers, respectively (Figs. 7A-D). Microbial levels after each dose of UV was applied varied between rivers and across sampling occasions. There were no detectable microbial colonies after 60 mJ.cm^{-2} across all rivers. The microbial counts for *Enterobacteriaceae*, ranged between 7 700 – 198 000, 160 – 100 000, 240 – 1 370 and 1 270 – 174 000 cfu.mL^{-1} for the rivers, respectively (Figs. 8A-D). Colony counts after UV radiation was applied was limited to one sampling occasion each of the Franschhoek and Mosselbank Rivers (Figs. 8C & 8D, respectively). The largest log reduction recorded was noted on sampling occasion four of the Mosselbank River, with a 5.2 log reduction, for Heterotrophic Plate Count colony counts. This was also the microbial test that had the highest initial colony count, of all the sampling occasions for all four rivers. A total of 1. 78 x 10^{6} cfu.mL^{-1} was recorded on this sampling occasion, before UV treatment was applied. This finding was coupled with increased TDS content and high turbidity readings (Table 2). This correlates with Chahal *et al.* (2016) that stated that increased turbidity values support the transport of attached pathogenic microorganisms, and therefore, increased treatment demands for disinfection.

It can be noted, that a more gradual reduction in the HPC population after UV radiation was recorded, than for the *Enterobacteriaceae* and *E. coli* populations, particularly in the Mosselbank River (Fig. 7D). This finding correlates with similar findings observed by Bester (2015) and Olivier (2015). The HPC population typically represents a broad spectrum of aerobic microorganisms, both Gram-positive and Gram-negative, that are able to grow on

Plate Count Agar (Oxoid, South Africa) at 30°C. When observing the HPC counts of the Mosselbank River, the prevalence of microorganisms after 20 and 40 $mJ.cm^{-2}$ of UV radiation was much higher than observed for any other river, and any other indicator population (*E. coli* and *Enterobacteriaceae*). These results could have a two-fold explanation. The overall initial microbial concentration in the Mosselbank River was higher than the other rivers (Fig 7A-D), therefore, even after a number of log reductions after UV radiation, the microbial count is still higher than the other rivers. Another possible explanation is that the type of microorganisms present in the microbial population in this river could be of a greater diversity, or show greater resistance to UV radiation than the organisms present in the other rivers, and therefore, result in their presence after 40 $mJ.cm^{-2}$ and 60 $mJ.cm^{-2}$ (Fig 7D). This could possibly be attributed to the effluent from the upstream water treatment plant releasing resistant bacteria into the river. These bacteria, which are clearly more resistant to UV than the *Enterobacteriaceae* and *E. coli* colonies, could show resistance to antimicrobial agents, too.

As discussed in the physico-chemical section, the suspended solids of a water sample reduce the efficacy of disinfection by shielding microorganisms or by the absorption of UV radiation (Abdul-Halim & Davey, 2016). The TSS and the UVT % of water samples were also reported to have an inverse relationship, which plays a role in the efficacy of UV disinfection. These factors should be taken into account when determining UV dosages. Water that has a lower turbidity, ultimately has higher UVT % readings and therefore, results in an improved disinfection efficacy. This is due to the fact that, much like suspended solids, microorganism aggregates can be enclosed within the particulates in the turbid samples, which increases the microorganism's resistance to UV penetration dramatically (Farrell *et al.*, 2018). Liu (2005) reported that an increase in turbidity from 1 to 10 NTU would reduce the average dosage by between 5 and 33%.

Based on the results obtained in the log reductions for indicator organisms after UV radiation, it can be noted that treatment efficacy is not impacted by water quality, however, the exposure time required to deliver the required dose is dramatically impacted. This has an impact on the efficiency or productivity of the user at farm-scale. A treatment step, possibly in the form of a filtration step, implemented before UV radiation to remove some organic matter could reduce exposure times required by improving the UVT %. The pathogen results obtained following UV radiation can be seen in Table 5.

Pathogen results after UV radiation

Table 5 Presence/absence results of *Listeria monocytogenes* (LM) and *Salmonella* spp. (S) for four rivers, over five sampling repetitions (rep.) after three UV radiation doses

		Rep. 1				Rep. 2				Rep. 3				Rep. 4				Rep. 5			
River	Test	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Plankenburg	*S*	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	
	LM	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	
Eerste	*S*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	
	LM	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	
Mosselbank	*S*	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	
	LM	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	
Franschhoek	*S*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	
	LM	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	

0 =No treatment, 1 = 20 mJ.cm⁻², 2 = 40 mJ.cm⁻², 3 = 60 mJ.cm⁻² of UV radiation
LM – *Listeria monocytogenes,* S – *Salmonella* spp.

Table 5 indicates the presence/absence results of the pathogen tests, before and after UV radiation at the specified doses. It is interesting to note that, on all but one occasion, the pathogens; *L. monocytogenes* and *Salmonella* spp. were no longer present after the lowest dose of UV radiation was applied. The presence of *L. monocytogenes* after 20 mJ.cm^{-2} in the Plankenburg River on the second sampling occasion might be attributed to a higher initial concentration of this organism in the river on this particular occasion. This is, however, a speculative observation as the detection methods used for both pathogens in this study are enrichment methods that do not allow for the determination of the actual microbial levels prior to enrichment. When observing the physico-chemical results on this day, there is no outlying factor or significantly differing characteristic that could contribute to this particular result. Due to the fact that the pathogens on other sampling events of this river, as well as the other three rivers were no longer present after the 20 mJ.cm^{-2} UV dose was applied (Table 5), it was concluded that this dose was mostly effective against the prevalent levels of pathogens in the rivers at the time of testing. The Franschhoek River had the lowest frequency of pathogen presence across the five sampling occasions. This correlates with the findings that this river had the best physico-chemical characteristics, of all four rivers. It can be noted, however, that the presence of *Salmonella* spp. on the fifth sampling occasion, was coupled with deteriorating water quality, including decreased UVT % and significant increases in TDS, EC, COD, turbidity and alkalinity (Table 2).

Gayán *et al.* (2014) stated that, in general, pathogenic bacterial strains are more resistant to UV radiation than non-pathogenic strains. A study performed by Gayán *et al.* (2012) on the effect of UV radiation on different *Salmonella* spp. with regard to their individual resistances, and noted that *Salmonella typhimurium* STCC 878 was the most resistant strain, requiring 18.03 $mJ.cm^{-2}$ to achieve a 4-log reduction. In the current study, it can be noted from the outlying result in the Plankenburg River, that when deciding on an effective dose to ensure complete microbial disinfection and water safety, that a dose of >20 $mJ.cm^{-2}$ may be required if pathogens are present at higher initial levels, or if more resistant strains are present.

Thirteen microbial strains were isolated from these tests and stored according to the procedure previously mentioned for further investigation in Chapter 4.

Conclusions

In this study, the utilisation of UV radiation as a method of disinfection of several river waters with varying water quality has, thus, been proven successful. The results of the indicator and pathogen testing in this study indicated that these rivers are not acceptable for agricultural use without pre-treatment. The indicator counts, specifically *E. coli,* notably exceeded the guideline limits on all sampling occasions. This means that transfer of *E. coli* from the water to fresh produce may be highly likely during agricultural irrigation. This presents a significant problem because pathogenic strains of *E. coli* and other pathogenic microorganisms may result in foodborne disease outbreaks following the consumption of contaminated produce. The baseline microbial and physico-chemical profiles of the rivers that were established, enabled the researcher to investigate the efficacy of UV disinfection over a broader range of rivers. A tentative correlation was made between the physico-chemical characteristics of a water sample and the microbial population levels, where deteriorating physico-chemical characteristics were coupled with higher microbial loads as well as a more consistent pathogen presence.

The Mosselbank River consistently had the poorest physico-chemical characteristics as well as the highest indicator population counts, and pathogen presence. This river could be considered as the ‘worst-case scenario’ for further UV treatment optimisation studies. If the dose requirements of UV radiation are based on ensuring that this river falls within the guideline specifications, there is a high probability that water from the other selected rivers will also be deemed safe after treatment.

The efficacy of UV radiation in the disinfection of microorganisms present in river water was confirmed in this study, where even at the lowest dose applied, the *E. coli* counts fell well within guideline limits. The HPC test results indicated a slower drop in microbial loads –

specifically in the Mosselbank River – which is indicative of a diverse and possibly more resistant microbial population in this river. *Enterobacteriaceae* levels decreased immediately after the lowest UV dose was applied. The recommended UV dose to ensure sufficient microbial disinfection for indicator organisms in rivers with similar profiles as those included in this study can thus be suggested to be between 20 – 40 $mJ.cm^{-2}$.

The pathogen results of this study indicate the efficacy of this treatment method against *Salmonella* spp. and *L. monocytogenes* after the lowest UV dose applied. Taking the outlying result observed in the Plankenburg River, where *L. monocytogenes* was present after 20 $mJ.cm^{-2}$ of UV radiation on one occasion into consideration, the recommended dose to ensure microbial disinfection for pathogenic microorganisms can, therefore, be suggested as between 20 – 40 $mJ.cm^{-2}$. The presence/absence testing of these pathogens does not provide an indication of the microbial load present. A recommendation would be to include a study on the pathogenic microorganisms isolated from the rivers at known initial concentrations to observe the response to UV radiation.

These recommendations, based on the results of the experiments in the current study, fall in line with USEPA (1999) suggesting that a dose of between 21-36 $mJ.cm^{-2}$ should be sufficient in the inactivation of bacterial and viral pathogens depending on water quality parameters such as turbidity and COD.

The organisms isolated and stored from these experiments will be utilised in Chapter 4 to determine the effect of an increased microbial concentration on UV disinfection efficacy, as well as to determine the resistance profiles between the river water isolates and isolates from other environmental and clinical sources.

Overall, the rivers examined in this study proved to be highly diverse considering their physico-chemical and microbial profiles. The results of the UV radiation experiments provide an indication that this treatment method is effective in ensuring microbial disinfection, and therefore, capable of providing a safe water supply for the irrigation of crops. It can, therefore, be concluded that UV radiation is effective at laboratory-scale and can be utilised to ensure river water disinfection of both indicator organisms as well as pathogenic microorganisms. This study provides sufficient information regarding the presence of selected microbial populations in the river waters as well as UV dose responses to facilitate an investigation into river water disinfection for use at farm-scale.

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Chapter 4

CHARACTERISATION OF RIVER WATER ISOLATES IN TERMS OF ULTRAVIOLET RESISTANCE, ANTIMICROBIAL RESISTANCE AND *LISTERIA MONOCYTOGENES* LINEAGE TYPING

Abstract

In this chapter, the antimicrobial resistance profiles of microbial strains isolated from the four rivers studied in the previous chapter (Chapter 3) were determined. The individual responses of the isolates to Ultraviolet (UV) radiation were also tested. The results provided insight into the microorganisms that currently exist in the rivers as well as the variances between river water isolates.

In Study A, the lineage type of the *Listeria monocytogenes (L. monocytogenes)* river water isolates was determined in order to predict the source of contamination. All river water isolates were classified as belonging to lineage I, which is associated with clinical infections and animals. This lineage is of great concern as it is responsible for over 95% of listeriosis infections worldwide.

In Study B, the antimicrobial resistance profiles of the river water isolates were determined against several antimicrobial agents. It was concluded that all *E. coli* (n = 3) and *Salmonella* spp. (n = 2) isolates were resistant to tetracycline, trimethoprim-sulfamethoxazole and ampicillin. A total of 80% of *L. monocytogenes* river water isolates (n = 4) were resistant to ampicillin, penicillin and erythromycin. Complete resistance (n = 5) was observed for *L. monocytogenes* isolates against trimethoprim-sulfamethoxazole. Multidrug resistance was reported in all but one of the river water isolates (n = 9). Extended Spectrum Beta-Lactamase (ESBL) testing indicated that none of the *Enterobacteriaceae* river water isolates were ESBL-producers.

In Study C, the same UV doses as studied in the previous chapter (Chapter 3) (20, 40 & 60 mJ.cm^{-2}) were utilised to determine the effect that a high initial microbial load (approximately 8 log cfu.mL^{-1}) may have on UV disinfection efficacy. The initial load was spiked to higher than what was found naturally in the river water. It was established that a UV dose of 60 mJ.cm^{-2} was not sufficient to reduce the *Escherichia coli* (*E. coli*) microbial load to acceptable levels (1 000 cfu.100 mL^{-1}). The Franschhoek River *E. coli* isolate was the most resistant strain tested, with an average cell concentration of log 5.2 after an UV dose of 60 mJ.cm^{-2} was applied. This is in comparison to the other four *E. coli* isolates, which presented an average cell concentration of log 3.1 following a dose of 60 mJ.cm^{-2}.

L. monocytogenes test results indicated that the Mosselbank River isolate was sensitive to UV radiation after 40 mJ.cm^{-2}, even at high initial microbial loads. The reference

L. monocytogenes isolates were more resistant to UV radiation compared to the Mosselbank River water isolate, as microbial presence was noted after the highest dose was applied to the reference strains. *Salmonella* species (spp.) river isolates were equally as sensitive to UV radiation than the other strains tested and required at least 40 $mJ.cm^{-2}$ of UV radiation for disinfection.

The resistance profiles and characteristics of the selected river water isolates were successfully determined in this study. The isolates carry resistance genes to multiple antimicrobials and therefore, pose a potential risk for human and animal health. It is evident that the microbial loads, as well as the food pathogen presence, in the rivers that have been studied in the Western Cape is a cause for concern as it could result in the contamination of fresh produce.

Introduction

In the Western Cape, South Africa, high levels of faecal contamination have been reported in river water intended for the irrigation of fresh produce (Britz *et al.,* 2013). This ongoing issue has resulted in the constant monitoring of rivers such as the Plankenburg and Eerste Rivers (Paulse *et al.,* 2012, Lamprecht *et al.,* 2014, Sivhute, 2019). The rivers analysed continuously exceeded the guideline limits for irrigation water (Department of Water Affairs and Forestry (DWAF), 1996), creating a risk for the consumers of the fresh produce irrigated with these waters. Investigations into effective methods of water disinfection for irrigation water have been performed, in rivers around the Western Cape in a number of studies (Schoeman *et al.*, 2013, Giddey, 2015, Olivier, 2015, Sivhute, 2019).

Listeria monocytogenes can be characterised by 13 different serotypes with four different lineages (Pirone-Davies *et al.,* 2018). The investigation into which lineage a particular isolate belongs to can provide an indication of a possible contamination source. It has been reported that lineage I isolates are responsible for over 95% of the listeriosis cases worldwide, and that lineage I isolates are, for instance, more virulent than lineage II.

Antibiotics have been one of the greatest discoveries of human history (Aslan *et al.,* 2018). However, a growing resistance to these antimicrobials raised concern in the clinical and agricultural industries, with over 20 000 resistance genes reported since 1930 (Aslan *et al.,* 2018). Blaak *et al.* (2015) states that contamination of surface water with bacteria that carry antimicrobial resistance genes may be as a result of animal defecation, agricultural run-off or sewer discharge. This surface water is then used for irrigation purposes, resulting in the contamination of crops with resistant bacteria, which are ingested by consumers. This poses a major public health concern, as antimicrobial resistant bacteria can cause infections that are

incredibly difficult to treat (Blaak *et al.,* 2015). Isolates were regarded as multidrug resistant (MDR) if they showed resistance to at least one antimicrobial in three or more classes (Basak *et al.,* 2016). The investigation into the presence of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* began when an increase in resistance to commonly-used antimicrobials – β-lactams – was reported (Shaikh *et al.,* 2015). These ESBL-producers synthesize an enzyme that is able to hydrolyse β-lactam antimicrobials that renders them inactive. The mutation of these β-lactamase enzymes causes resistance to even newly developed β-lactam antimicrobials (Shaikh *et al.,* 2015). Blaak *et al.* (2015) states that an *Enterobacteriaceae* strain that contains one or more ESBL-genes, can disseminate those genes into the environment.

Ultraviolet (UV) radiation has been successfully used as a water treatment method, specifically for drinking water since the 1980s in Europe (Hijnen *et al.,* 2006). This method of disinfection gained interest after the discovery that it is effective against *Cryptosporidium* spp. and *Giardia* spp., both of which have shown resistance to chlorine (Hijnen *et al.,* 2006). Gayán *et al.* (2014) states that larger-sized cells may provide greater resistance to UV radiation than smaller ones. This is due the fact that there may be an increase in the probability of the photons of UV radiation being absorbed by components in the cell other than the DNA. This is the reason for yeasts and moulds requiring higher doses for inactivation (Gayán *et al.,* 2014). Investigations into the efficacy of this method of disinfection have normally occurred at laboratory-scale, with pure cultures of *E. coli* strains (Zimmer *et al.,* 2007). No research, specifically in South Africa, has been reported for the use of UV radiation to disinfect naturally occurring environmental isolates of food pathogens such as *L. monocytogenes* and *Salmonella* spp. present in irrigation water.

In the previous chapter (Chapter 3), four rivers were investigated to determine the microbial and physico-chemical profile over the summer irrigation period. *Escherichia coli, L. monocytogenes* and *Salmonella* spp. isolates were obtained from the rivers. It was established from the microbial and physico-chemical characteristics of the rivers under investigation, that these rivers were unacceptable for agricultural irrigation without pre-treatment, as all exceeded the irrigation water guideline limit of 1 000 cfu of *E. coli* per 100 mL (DWAF, 1996). The consistent presence of food pathogens such as *Salmonella* spp. and *L. monocytogenes* further raised concern for the safety of consumers of fresh produce irrigated with this water. A deeper investigation into the specific microbial resistance profiles of these isolates could provide a more complete understanding of the potential health risk that the level of contamination in these rivers pose.

In this three-part study, the microbial characteristics of these river isolates were analysed, including their responses to UV radiation and antimicrobial resistance profiles.

Reference strains from multiple sources were included for comparative purposes.

Research Design

This chapter is divided into three separate studies. The first involves the analysis of the *L. monocytogenes* river isolates through Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) to determine the lineage type of each isolate. The second study investigates the antimicrobial resistance profiles of all isolates and ESBL presence of *Enterobacteriaceae* isolates. The final study investigates of the effect of low-pressure (LP) UV radiation on selected bacterial isolates at high initial concentrations using three UV doses.

Study A *– Characterisation of L. monocytogenes isolates from river sources using PCR-Restriction Fragment Length Polymorphism.*

L. monocytogenes is said to be ubiquitous in the natural environment (Pirone-Davies *et al.,* 2018). Environmental strains of *L. monocytogenes* isolates have shown to belong to any one of four lineages (Pirone-Davies *et al.,* 2018). The objective of Study A was to determine the lineage type of each of the *L. monocytogenes* isolates which are listed in Table 1. PCR analysis allows for the confirmation of the species, which is observed after viewing the gel following electrophoresis. Following this step, PCR-restriction fragment length polymorphism (RFLP) is performed to determine the lineage type of each isolate. The lineage of *L. monocytogenes* enables for predictions of the source of contamination to be made. The RFLP method of lineage type characterisation has been described by Rip & Gouws (2020).

Study B *– ESBL and Antimicrobial Susceptibility Testing of isolates.*

The purpose of Study B was to screen the *Enterobacteriaceae* isolates for the presence of ESBL producers, as well as to determine the antimicrobial resistance profiles of all isolates (including *L. monocytogenes* isolates) (Table 1). The standard test procedures were followed as set out by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020) for ESBL testing and the Clinical Laboratory Standards Institute (CLSI, 2016) for antimicrobial susceptibility testing. Both ESBL and antimicrobial susceptibility testing was performed using the disc diffusion method. The choice of the antimicrobials that were included in this study was based on literature, and comprised of commonly used antimicrobials in the agricultural and clinical sectors. These procedures were performed twice per strain. Strain viability confirmation and preparation for testing are described in the general materials and methods section. An organism is considered to be an ESBL-producer if the inhibition zone diameters are ≥5 mm larger with clavulanic acid than the discs without clavulanic acid (EUCAST, 2020). An organism is considered to be resistant to an antimicrobial agent if the

inhibition zone diameters are smaller than the prescribed diameter, and susceptible to that antimicrobial if the inhibition zone diameter is larger than the prescribed diameter (EUCAST, 2020).

Study C *– Comparison of resistance profiles of selected river water isolates to reference strains using low-pressure UV radiation at high initial microbial loads.*

In Study C, the resistance profiles of selected river water isolates were compared to the resistance profiles of certain American Type Culture Collection (ATCC), clinical and environmental strains of the same species, against low-pressure (LP) UV radiation. This study also served to determine the efficacy of three doses of UV radiation for disinfection against an increased microbial concentration in autoclaved river water. The river water isolates and reference strains utilised in this study are listed in Table 1. Each microbial strain was tested three times.

Methods and Materials

This chapter is divided into three separate studies. The following section is divided into two parts, which includes the general materials and methods of the three studies and then the research design summary of each study.

General Materials and Methods

Microbial cultures

In the previous chapter (Chapter 3), isolates were obtained from the river water (before and after UV radiation) by streaking a single colony from the respective selective agar plates onto Nutrient Agar (Oxoid, South Africa) and incubating for 24 hours at 37°C. A single colony from the Nutrient Agar plate was inoculated into 5 mL Tryptone Soya Broth (TSB) (Oxoid, South Africa) and incubated for a further 24 hours at 37°C. Following this incubation period, 800 µL of this TSB-bacterial suspension was placed in a sterile Cryovial with 800 µL of sterile 50 % ($v.v^{-1}$) glycerol solution and gently mixed. This resulted in a bacterial stock solution with a final concentration of 25% ($v.v^{-1}$) glycerol, which was stored at -80°C until future use. The isolates are listed in Table 1, along with sampling dates and UV treatments applied before isolation (where applicable).

Confirmation of strain viability

Prior to each experiment, the viability of test isolates was confirmed by streaking onto the appropriate selective agars. This was achieved by inoculating 100 µL of bacterial suspension that had been stored in glycerol (25 % $v.v^{-1}$) at -80°C into 5 mL sterile tryptone soy broth (TSB) (Oxoid, South Africa) and incubating at 37°C for 24 hours. A loop full of the each of the *E. coli* inoculums were then streaked out onto Levine's Eosin Methylene-Blue Lactose Sucrose agar (L-EMB) (Oxoid, South Africa) and incubated at 36°C for 24 hours. The presence of *E. coli* was confirmed with metallic green-coloured colonies on the L-EMB agar. A loop full of each of the *Salmonella* spp. strains was streaked onto Xylose Lysine Deoxycholate Agar (XLD) (Oxoid, South Africa) and inversely incubated at 37°C for 24 hours. The appearance of red colonies with black centres indicated a positive test for *Salmonella* spp. A loop full of each of the suspensions of *L. monocytogenes* strains was streaked onto Rapid'*L.mono* Agar plates (BioRad, South Africa) and incubated at 30°C for 24 hours. A positive test for *L. monocytogenes* was considered to be the appearance of blue/black colonies on the agar after incubation.

Table 1 Microorganisms utilised in the three studies of Chapter 4, including their source, known antimicrobial resistance profiles and UV dose applied before isolation

Isolate code	Source & isolation date	Known resistance	Treatment applied	Study used
E. coli FR-01	Franschhoek River (16/10/2019)	None	20 mJ.cm^{-2} UV	B & C
E. coli FR-02	Franschhoek River (09/10/2019)	None	20 mJ.cm^{-2} UV	B & C
E. coli MR-01	Mosselbank River (22/01/2020)	None	20 mJ.cm^{-2} UV	B & C
E. coli CTX-TEM	Fresh produce	aminoglycosides, flouroquinolones, trimethoprim-sulfamethoxazole,	None	B & C
E. coli BAA-2469	ATCC BAA-2469 (NDM +)	All – except nitrofurantoin & tigecycline	None	B & C
E. coli ATCC 35218	ATCC 35218	ampicillin, penicillin, Cephalosprins	None	B & C
Salmonella MR-02	Mosselbank River (22/01/2020)	None	None	B & C
Salmonella PR-02	Plankenburg River (22/01/2020)	None	None	B & C
Salmonella – poultry	Poultry source	None	None	B & C
S. braenderup	*S. braenderup* H9812 (clinical source)	None	None	B & C

Table 1 Continued

Isolate code	Source & isolation date	Known resistance	Treatment applied	Study used
L. monocytogenes PR-03	Plankenburg River (22/01/2020)	None	20 mJ.cm^{-2} UV	A & B
Listeria MEN09	MEN-09 (Lineage I – food production equipment)	tetracycline, erythromycin, gentamycin	None	A & B & C
Listeria MEN32	MEN-32 (Lineage II – raw beef)	tetracycline, erythromycin, gentamycin	None	A & B
ATCC 23074	ATCC 23074	None	None	B & C
L. monocytogenes FR-01	Franschhoek River (25/10/2019)	None	None	A & B
L. monocytogenes FR-02	Franschhoek River (25/10/2019)	None	None	A
L. monocytogenes FR-03	Franschhoek River (22/01/2020)	None	None	A
L. monocytogenes MR-01	Mosselbank River (25/10/2019)	None	None	A & B
L. monocytogenes MR-03	Mosselbank River (22/01/2020)	None	None	A & B & C
L. monocytogenes PR-01	Plankenburg River (16/10/2019)	None	None	A & B
L. monocytogenes PR-02	Plankenburg River (08/11/2019)	None	None	A

Identification of isolates using MALDI-TOF

Following isolation from agar plates, the isolates were purified and prepared for species identification with a MicroFlex LT Matrix-Assisted Laser Desorption/ Ionisation Time of Flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Germany). The MALDI Biotyper 3.0 software was utilised to determine the identity of each isolate by comparing spectra with reference strains in the database. The similarity of a reference strain to the tested isolate was correlated using a logarithmic score, which was interpreted according to the manufacturer's guidelines (Zulu, Z. 2020, Researcher, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria). A log value of ≥ 2.300 indicates the species identification with a high level of confidence. An intermediate log value score of ≥ 2.000 lindicates a probable species identifcation. A log value of between 1.700 and 1.999 indicates only the identification of a genus. Any scores below 1.700 does not allow for any identification to be made. This is often as a result of a mixed culture being present, or that the isolate under investigation is not present in the current database and therefore cannot be idenitified (Zvezdanova *et al.,* 2020). The results of these anaylses can be seen in Tables 8 and 9.

Polymerase Chain Reaction and RFLP analysis

Isolates, listed in Table 1 for Study A, were removed from the -80°C freezer, and 20 µL of each isolate was inoculated into 10 mL of autoclaved TSB (Oxoid, South Africa). This was incubated at 37°C for 24 hours. The isolation of DNA was achieved by following the protocol set out in the Zymo Research DNA Extraction Kit (Inqaba Biotec, South Arica). The resulting isolated and purified DNA in dissolved form was stored according to the protocol for the PCR analysis.

Polymerase Chain Reaction

The PCR analysis was performed using the method which was described by Rip & Gouws (2020). The PCR process was performed in order to confirm that the river water isolates were in fact *L. monocytogenes* prior to enzyme digestion. The *hly* specific primers were selected for this experiment (IDT Technologies, South Africa). This gene is responsible for the virulence of *L. monocytogenes* as it encodes for listeriolysin O. This pore-forming cytotoxin exhibits haemolytic activity (De Mello *et al.,* 2008). The primer sequences utilised can be seen in Table 2. The PCR amplification reaction was performed using 25 µL volumes. The Multiplex TEMPase 2x Master Mix (Lasec, South Africa) was utilised for these experiments. This Master Mix consisted of TEMPase Hot Start DNA Polymerase, 0.4 mM of each dNTP and 3.0 mM $MgCl_2$ (at 1X). The 25 µL amplification reaction consisted of 12.5 µL Master Mix, 10.5 µL RNA free water (Inqaba Biotechnology, South Africa), 0.5 µL of each of the primers (final concentration of 0.2 µM) and 1 µL of the previously extracted DNA (Rip & Gouws, 2020). A positive and negative control was included in this experiment. The negative control consisted

of nuclease free water, and the positive control was a *L. monocytogenes* MEN 09 lineage I isolate (Rip, D. 2020, Lecturer and Researcher, Stellenbosch University, Stellenbosch, personal communication, 28 January). The thermal cycler utilised for this experiment was the T100 Thermal Cycler (BioRad, South Africa). The conditions selected were: initial denaturation for 3 min at 94°C followed by 30 cycles of denaturation at 94°C for 40 s. Primer annealing was performed at 55°C for 40 s, and extension took place at 72°C for 40 s. The final extension lasted for 5 min at 72°C (Rip & Gouws, 2020). The PCR products were then cooled to 4°C and stored at -18°C until electrophoresis was performed for visualisation. The process of DNA extraction, PCR amplification and gel electrophoresis was completed twice.

Table 2 Forward and reverse primer sequence utilised for *L. monocytogenes* PCR testing (Rip & Gouws, 2020)

Oligosaccharide	Sequence (5' – 3' orientation)
Forward primer - hlyF	CAT TAG TGG AAA GAT GGA ATG
Reverse primer - hlyR	GTA TCC TCC AGA GTG ATC GA

Electrophoresis visualisation

A 2% agarose gel was prepared that contained 10 000 x EZ-Vision® Bluelight DNA dye (VWR, South Africa). The wells were loaded with 6X TriTrack DNA Loading Dye (Thermo Scientific, South Africa). The PCR amplicon size was compared to a 100 base pair DNA ladder (Thermo Scientific, South Africa). The DNA fragment separation conditions were 85 V for 120 minutes. The DNA fragments were visualised using the Bio-Rad Gel doc XR+ System (Bio-Rad, South Africa), which was combined with Image Lab Software version 5.2.1.

RFLP Analysis

Once the isolates, listed in Table 1, were confirmed as *L. monocytogenes* from the above electrophoresis step, the remaining PCR products obtained from the PCR amplification process were utilised for the RFLP analysis. Following the method described by Rip and Gouws (2020), a 10 µL restriction digest was made, which contained 5 µL of the PCR product, 2.5 µL RNA free water, 1.5 µL Fast Digest *NdeI* Enzyme (Thermo Scientific, South Africa) and 1 µL 1X final concentration restriction buffer (Thermo Scientific, South Africa). This enzyme will cut lineage I isolates only (Rip & Gouws, 2020). This was allowed to digest for 25 min at 37°C, and then immediately placed on ice to halt the reaction. A positive and negative control were included in this reaction. The positive control used was *L. monocytogenes* MEN09 lineage I isolate (Rip, D. 2020, Lecturer and Researcher, Stellenbosch University, Stellenbosch, personal communication, 28 January). The negative control used was an uncut DNA sample obtained from a PCR product of an isolate before digesting with the enzyme. The

procedure for electrophoresis visualisation was then followed, as described above. However, the conditions for separation were 80 V for 120 minutes. The RFLP procedure was completed twice. If the characteristic band sizes were not observed for lineage I from the isolates tested, the procedure described above would have been repeated with the Fast Digest *HaeII* enzyme under different digestion conditions (Thermo Scientific, South Africa) for lineage II isolates.

Preparation of strains for ESBL and antimicrobial susceptibility testing

Bacterial isolates used in this section are indicated in Table 1. Bacterial strains (stored in 25% $v.v^{-1}$ glycerol) were removed from the -80°C freezer, defrosted, and a 20 µL inoculum was suspended in five mL of sterile TSB and was then incubated for 24 hours at 37°C. Following viability confirmation on respective selective agars, *Salmonella* and *E. coli* strains were streaked onto Nutrient Agar (NA) (Oxoid, South Africa) and inversely incubated at 37°C for 24 hours. The *L. monocytogenes* strains were streaked onto Brain Heart Infusion agar (BHI) (Oxoid, South Africa) and incubated inversely at 37°C for 24 hours.

McCartney bottles containing 25 mL sterile distilled H_2O ($_dH_2O$) were inoculated with a single colony from each respective agar plate (NA and BHI) to reach an approximate cell density correlating to the 0.5 McFarland Standard (BioMèrieux, South Africa). A confirmatory test for bacterial cell optical density (OD) was then performed using a Spectroquant Prove 600 Spectrophotometer (Merck, South Africa), zeroed using sterile distilled water. The absorbance of each suspension was then measured at 600 nm, and adjusted to ensure a final absorbance reading of 0.2 was achieved. This procedure remained the same for both ESBL and antimicrobial susceptibility testing.

ESBL testing procedure

For ESBL testing of the *Enterobacteriaceae* strains, Mueller-Hinton agar for non-fastidious organisms (Oxoid, South Africa) was prepared in duplicate for each strain, before it was inoculated using a sterile cotton swab, that had been soaked in the respective suspensions. Excess fluid was removed from the swab by pressing it against the walls of the swab tube. Inoculation was performed by moving the swab in horizontal, vertical, and diagonal directions to ensure that the surface was completely covered to form a consistent lawn over the agar (EUCAST, 2020). Using a disc dispenser (Thermo Scientific, South Africa), discs of ceftazidime [30 µg], cefotaxime [30 µg] and cefepime [30 µg], each alone and in combination with clavulanic acid [10 µg] were dispensed onto each plate, in duplicate for each strain (Davies Diagnostics, South Africa). These plates were then inversely incubated for 24 hours at 37°C. Zone diameters were then measured with a sterile ruler. *Enterobacteriaceae* strains are deemed to be ESBL producers if the inhibition zone diameter is ≥ 5 mm larger with the clavulanic acid containing discs compared to the discs without clavulanic acid (EUCAST,

2020). *Klebsiella pneumoniae* 700603 was included as a positive control during ESBL-testing, as stipulated in the EUCAST (2020) procedure.

Antimicrobial Susceptibility Testing procedure

Antimicrobial susceptibility testing (AST) was then performed on the same strains tested for ESBL production, again using the disc diffusion method and included the *L. monocytogenes* strains (Table 1) (CLSI, 2016, EUCAST, 2020). For *Enterobacteriaceae,* Mueller-Hinton agar, for non-fastidious organisms, was prepared and then inoculated in the same manner as described for the ESBL testing. A disc dispenser (Thermo Scientific, South Africa) was then utilised to dispense antimicrobial discs onto the surface of the plate. Each strain was tested in duplicate. The antimicrobial discs (Thermo Scientific, South Africa) used for *E. coli* and *Salmonella* spp. strains are presented in Table 3. The 15-15-15 minute rule was followed according to the procedure set out by EUCAST, (2020). This rule can be described as the amount of time (15 min) suggested for culture suspension (0.5 McFarland standard), another 15 min to apply the antimicrobial discs, and the final 15 min for the time allotted to place the plates in incubator after disc application. Plates were inversely incubated for 18 hours at 37°C, and then inhibition zone diameters were measured using a ruler. *E. coli* ATCC 35218 and *E. coli* ATCC 25922 were included as positive and negative controls, respectively, for antimicrobial resistant *Enterobacteriaceae.* The *E. coli* ATCC 35218 strain has a known resistance to ampicillin and other antibiotics. According to EUCAST (2020), this is as a result of the presence of the TEM-1 β-lactamase enzyme. The *E. coli* ATCC 25922 strain is susceptible to most antibiotics.

Table 3 Antimicrobials used in this study for the testing of antimicrobial resistance in *Enterobacteriaceae* isolates

Antibiotic class	Antibiotic (& abbreviation)	[Disc] (µg)
Penicillin	Ampicillin (AMP 10)	10
Chloramphenicol	Chloramphenicol (C 30)	30
Fluoroquinolones	Ciprofloxacin (CIP 5)	5
Aminoglycoside	Gentamycin (GM 10)	10
	Streptomycin (S 10)	10
Sulphonamide	Trimethoprim-sulfamethoxazole (STX 25)	25
Tetracycline	Tetracycline (TE 30)	30
Quinolone	Nalidixic acid (NA 30)	30

Nalidixic acid and streptomycin were not prescribed in the EUCAST (2020) or CLSI (2016) manuals for antimicrobial susceptibility testing of *Enterobacteriaceae*. The inclusion of these two antimicrobials was based the multitude of literature sources that included these antimicrobials in the testing specifically of *Salmonella* spp. (Mensah *et al.,* 2019, Kebede *et al.,* 2016, Maguire *et al.,* 2019, Revolledo & Ferrerira, 2010). These two antimicrobials, as well as the others indicated in Table 3, are routinely used in the investigation of antimicrobial resistances in *Salmonella* spp. isolated from environmental sources. Nalidixic acid and streptomycin discs were only used in *Salmonella* spp. isolate testing and were not used in *E. coli* isolate testing.

Antimicrobial susceptibility testing on *L. monocytogenes* isolates was performed on Mueller-Hinton Agar with a supplement of 5% defibrinated horse blood and 20 mg. L^{-1} β-NAD (MH-F) (Thermo Scientific, South Africa). These plates are suitable for growing this particular fastidious microorganism. These pre-set plates were inoculated in duplicate in the same manner as the *Enterobacteriaceae,* however, different discs (Thermo Scientific, South Africa) were utilised (Table 4), based on EUCAST (2020) procedure. Again, the 15-15-15 minute rule was followed. Gentamycin was not included in the EUCAST (2020) procedure, however, this antimicrobial is routinely used in the determination of antimicrobial resistances of *L. monocytogenes* isolates obtained from clinical and environmental sources (Lyautey *et al.,* 2007, Caplan *et al.,* 2014). The combination of ampicillin and gentamycin, or just ampicillin alone is commonly used in clinical practices as the first-line drugs of choice for listeriosis treatment (Chen *et al.,* 2020). *Streptococcus pneumoniae* ATCC 49619 was utilised as the control strain in this experiment (EUCAST, 2020). All plates were inversely incubated for 18 hours at 37°C, and then inhibition zone diameters were measured using a ruler.

Table 4 Antimicrobial discs used in *L. monocytogenes* isolates resistance testing

Antibiotic class	Antibiotic (& abbreviation)	[Disc] (µg)
Penicillin	Ampicillin (AMP 2)	2
	Penicillin (P 1)	1
Carbapenems	Meropenem (MEM 10)	10
Macrolides	Erythromycin (E 15)	15
Aminoglycoside	Gentamycin (GM 10)	10
Sulphonamide	Trimethoprim-sulfamethoxazole (STX 25)	25

The breakpoint zone diameters (mm) were measured after incubation, and the diameters were compared to the EUCAST Breakpoint Tables for *Enterobacteriaceae* and for *L. monocytogenes* (EUCAST, 2020). If the inhibition zone diameter is larger than the prescribed

diameter, that isolate is deemed to be susceptible to treatment with that antimicrobial. If the inhibition zone diameter is smaller than the specified diameter, that isolate is deemed to be resistant to that particular antimicrobial (EUCAST, 2020). All isolates were tested in duplicate, and this procedure was performed twice per isolate. The breakpoint zone diameters are presented in Tables 5 and 6.

Table 5 Break points diameters for antimicrobial agents for *Enterobacteriaceae* used in this study (CLSI, 2016, EUCAST, 2020)

Antimicrobial	Abbreviation	Breakpoint zone diameter (mm)	
		S >	R <
Ampicillin	AMP	14	14
Chloramphenicol	C	17	17
Ciprofloxacin	CIP	25	22
Gentamycin	GM	17	17
Streptomycin	S	24	23
Trimethoprim-sulfamethoxazole	STX	14	11
Tetracycline	TE	15	11
Nalidixic Acid	NA	18	17

S – Susceptible; R – Resistant

Table 6 Breakpoint zone diameters for antimicrobials used for *L. monocytogenes* isolates tested in this study (CLSI, 2016, EUCAST, 2020)

Antimicrobial	Abbreviation	Breakpoint zone diameter (mm)	
		S >	R <
Ampicillin	AMP	13	13
Penicillin	P	16	16
Meropenem	MEM	26	26
Erythromycin	E	25	25
Gentamycin	GM	19	19
Trimethoprim-sulfamethoxazole	STX	29	29

S –Susceptible; R – Resistant

Preparation of microbial cultures for UV radiation

All river water isolates and standard reference cultures (Table 1) were stored in glycerol (25% $v.v^{-1}$) at -80°C until required. Bacterial strains were prepared for UV radiation in the following manner. Each strain was removed from the -80°C freezer, defrosted, and a 100 µL inoculum was suspended in 10 mL sterile TSB and was then incubated for 24 hours at 37°C. Following viability confirmation (as described in the previous section), *Salmonella* strains were streaked onto Nutrient Agar (NA) (Oxoid, South Africa) and inversely incubated at 37°C for 24 hours. The *Salmonella* river water and reference strains utilised in this experiment can be seen in Table 1. The *L. monocytogenes* strains were streaked onto Brain Heart Infusion Agar (BHI) (Oxoid, South Africa) and incubated inversely at 37°C for 24 hours. The *L. monocytogenes* reference and river water strains used in this experiment are summarised in Table 1. The *E. coli* isolates were utilised directly from a 24 h incubated inoculum in TSB. The *E. coli* reference and river water isolates utilised in this experiment can be seen in Table 1.

Escherichia coli *isolate preparation for UV radiation*

One mL of the 10 mL *E. coli* inoculum was removed using a sterile pipette and added to a sterile 1.5 mL Eppendorf tube. This procedure was repeated five times per strain. All five Eppendorf tubes were then centrifuged using a Sigma 1-14 Microfuge (Sigma, South Africa), for one minute at 10 000 x *g.* The supernatant of each tube was removed and the pellet was re-suspended in one mL of autoclaved Plankenburg River water, and vortexed to ensure a uniform mixture. Standardisation was performed by aseptically transferring one mL of bacterial suspension to a sterile cuvette. The bacterial cell optical density (OD) was performed using a Spectroquant Prove 600 Spectrophotometer (Merck, South Africa), zeroed using the same autoclaved river water as was used for the suspensions. The absorbance was then measured at 600 nm, to ensure an absorbance reading of 0.2 was achieved. This one mL of *E. coli* inoculum that was used in the spectrophotometer was discarded, and the remaining four mL was utilised in the experiment. These aliquots came from the same initial suspension, which was adequately vortexed, so all had the same concentration. Only one mL of sample is required for *E. coli* testing for each treatment applied (i.e. per dilution series). As there are four treatments, i.e. before treatment, and three different UV doses, a total of four mL is required. The contents of the first Eppendorf was then placed into nine mL of sterile buffered peptone water (BPW) in a test tube for the dilution series for before UV treatment. The contents of the remaining three Eppendorf tube (total of three mL) were then transferred to a sterile petri dish for UV treatment. This method was adapted from a similar method by Zimmer & Slawson (2002).

Salmonella *spp. and* L. monocytogenes *preparation for UV radiation*

For *Salmonella* spp. and *L. monocytogenes* UV susceptibility testing, the preparation process for UV treatment remained the same for both bacteria and is described as follows. On the day of experimental testing, a single colony from each respective agar plates (following a 24 h incubation) was selected using a sterile loop and transferred to 100 mL autoclaved Plankenburg River water in a Schott bottle to yield a cell density equivalent to a 0.5 McFarland standard (BioMèrieux, South Africa). The test for bacterial cell optical density (OD) was performed using a Spectroquant Prove 600 Spectrophotometer (Merck, South Africa), in the same manner as the *E. coli* testing. The quantity of 100 mL was prepared as each dose (including before treatment) requires 25 mL of bacterial suspension. This is due to the fact that *L. monocytogenes* and *Salmonella* spp. testing requires 25 mL of sample for the enrichment step (ISO method 11290-1, 2017 & SANS method 19250, 2011). As there are four treatments, i.e. before treatment, and then three different UV doses, a total of 100 mL was required per strain. This method was adapted from a similar method by Zimmer & Slawson (2002).

Ultraviolet treatment of bacterial strains

Prior to UV treatment, the Ultraviolet Transmission percentage (UVT %) of each prepared bacterial suspension was measured using the Sense T254 UV Transmission (%) Photometer (Berson, Netherlands). The light intensity of the UV lamp was measured with the ILT1400 radiometer (International Light Technologies, USA) which is coupled with a XRL140T254 detector (International Light Technologies, USA).

The exposure time necessary to apply each dose was calculated, using the known sample depth. For the pathogen testing (*Salmonella* spp. & *L. monocytogenes*), an amount of 25 mL of the bacterial strain was removed from the Schott bottle for the microbial test before treatment was applied, leaving 75 mL of the bacterial strain for the three doses of UV radiation (3 x 25 mL). The remaining 75 mL in Schott bottles was then transferred to a sterile beaker containing a sterile magnetic stirrer. Each bacterial strain was then subjected to UV radiation at the three doses (20, 40 & 60 $mJ.cm^{-2}$) while being stirred with the magnetic stirrer, on a medium speed. For *E. coli* testing, one mL was required per dose of UV to perform the dilution series for analysis. Following each UV dose application, the respective microbial tests were performed. This process is described in Fig. 1. Due to experimental challenges, *L. monocytogenes* MR-03 was not tested with the same batch of Plankenburg River water as the rest of the isolates. *L. monocytogenes* ATCC 23074 was tested in both samples of Plankenburg River water in order to determine the effect of the physico-chemical changes of the two river water batches on the treatment efficacy. The physico-chemical characteristics of the river water used for these two isolates are listed in Table 7 for Test 2. The physico-chemical

characteristic results of the sterile Plankenburg River water used for the testing of the remaining isolates in Study C can be seen in Table 7 under Test 1. The methods for the physico-chemical analysis can be seen in Chapter 3.

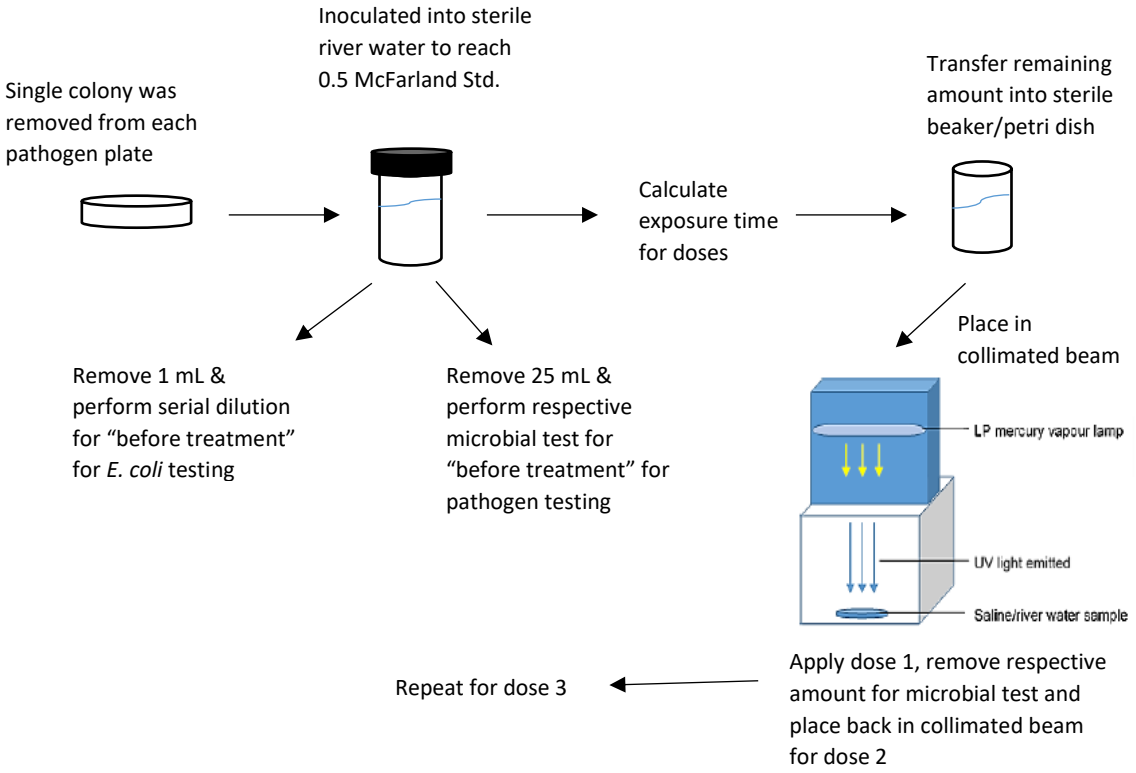

Figure 1 Experimental design of Study C for the application of three doses of UV radiation to bacterial isolates

Table 7 Physico-chemical analysis of sterile Plankenburg River water utilised in Study C

Physico-chemical parameter	Result Test 1	Result Test 2
UVT %	59.7	80
TDS ($mg.L^{-1}$)	123	176
EC ($mS.m^{-1}$)	0.16	0.22
COD ($mg\ O_2.L^{-1}$)	14	12
Turbidity (NTU)	15	7.01
TSS ($mg.L^{-1}$)	13	9
pH	7.17	7.08
Alkalinity ($mg.L\ CaCO_3^{-1}$)	80	70

Salmonella spp. detection

In order to determine the efficacy of UV treated *Salmonella* spp., 25 mL from each treated *Salmonella* sample was inoculated into 225 mL sterile BPW and incubated at 30°C for 24 hours. Following incubation, 0.1 mL of this solution was inoculated into 10 mL of sterile Rappaport Vassiliadis (RV) broth (Oxoid, South Africa), and incubated at 42°C for 24 hours. A loop full of each inoculum was then streaked onto XLD (Oxoid, South Africa) plates in duplicate, using a sterile loop, and inversely incubated at 37°C for 24 hours (SANS 19250, SANS, 2011). *Salmonella* spp. was indicated by the presence of red colonies with a black centre the agar. This process was repeated three times per strain.

L. monocytogenes detection

Each *L. monocytogenes* strain was treated with the specified UV doses, after which 25 mL of each sample was inoculated into 225 mL sterile ½ Fraser Broth containing a *Listeria* selective supplement (Oxoid, South Africa). This was incubated at 30°C for 24 hours. Following this, a sterile loop was then used to streak each strain, in duplicate, onto Rapid'L.mono Agar plates (BioRad, South Africa), and incubated at 37°C for 24 hours (ISO Method 11290-1, 2017). *L. monocytogenes* was indicated by the presence of blue/black coloured colonies. This process was repeated three times per strain.

Escherichia coli detection and enumeration

After each *E. coli* strain was exposed to the UV light (using the same doses applied to the *Salmonella* and *L. monocytogenes* strains), one mL of the suspension was removed from the petri dish and inoculated into a test tube containing nine mL of sterile BPW and a dilution series was prepared up to a dilution of 1×10^{-6}. This was repeated after the application of doses two and three. This process was also performed before treatment to determine initial microbial loads. One mL of each dilution was then plated with the pour plate technique, in duplicate, with approximately 20 mL of Brilliance Chromogenic *E. coli*/ Coliform Agar (Oxoid, South Africa) and inversely incubated at 37°C for 24 hours (ISO Method 16654:2001). Purple coloured colonies between 10 and 300 were counted. This process was repeated three times per strain.

A summary of the three studies in this chapter can be seen in Figure 2.

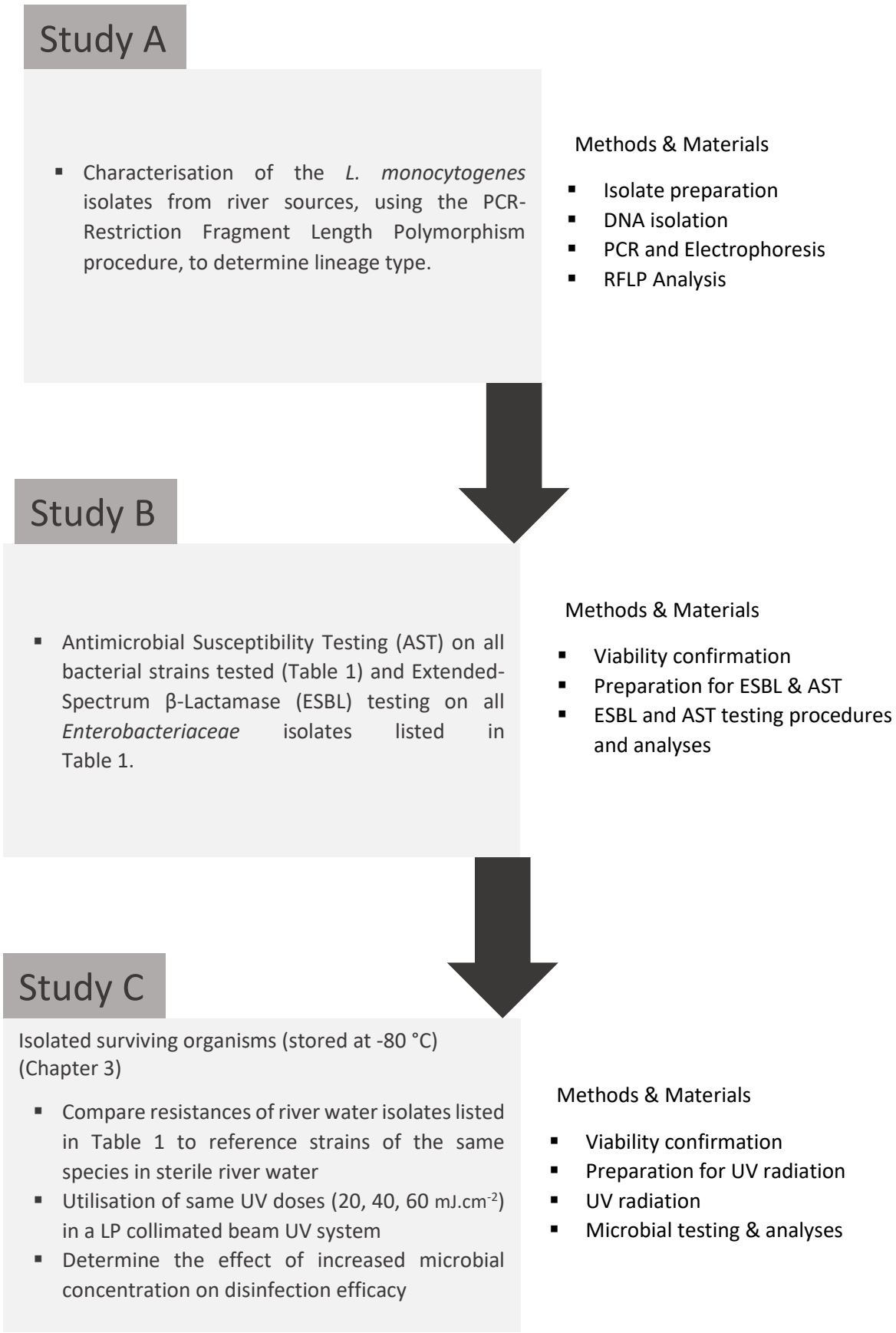

Figure 2 Summary of research design of three studies in Chapter 4

Results and Discussion

***Study A**: RFLP Characterisation of L. monocytogenes isolates*

Pirone-Davies *et al.* (2018) reports that infection from *L. monocytogenes* is difficult to control due to the widespread dissemination of this organism in the natural environment. Chen *et al.* (2017) indicates that *L. monocytogenes* is capable of surviving normal as well as severe environmental conditions, which adds to the complexity of this organism. *L. monocytogenes* has been isolated from a wide variety of environmental sources, including food products, river water, industrial effluent, soil, human faeces and animals (Chen *et al.,* 2017). *L. monocytogenes* can be divided into four phylogenetic lineages, which vary in their evolutionary, ecological and virulence characteristics. The determination of lineage type provides an indication of the source of contamination (Chen *et al.,* 2017).

L. monocytogenes consists of four different lineages (Chen *et al.,* 2017). Within these four lineages, are thirteen recognised *L. monocytogenes* serotypes (Pirone-Davies *et al.,* 2018). These thirteen serotypes are based on the flagellar (H) and somatic (O) antigens (Jamshidi & Zeinali, 2019). Lineage I includes the following serotypes; 1/2b, 3b, 3c and 4b. Lineage II includes 1/2a, 1/2c and 3a and lineage III serotypes includes 4a and 4c (Chen *et al.,* 2017). Listeriosis is predominantly caused by the 1/2a (lineage II), 1/2b (lineage I) and 4b (lineage I) serotypes, and are reportedly responsible for over 95% of clinical cases (Burall *et al.,* 2017).

In order to determine the lineage of the river isolates in this study, the first step was to perform PCR analysis on all river water isolates for species confirmation. Following confirmation, isolates were subjected to PCR-RFLP analysis to determine lineage types. The procedure set out by Rip and Gouws (2020) was followed. The PCR and RFLP procedures were performed in duplicate for each strain. The results from the repeated experiment were identical.

All isolates were confirmed as *L. monocytogenes* with PCR analysis, where the *hly*F and *hly*R genes (730 base pairs fragment) was analysed, which represents the undigested amplification product. These isolates were then analysed for lineage type using the RFLP method set out by Rip and Gouws (2020).

Following species confirmation, the PCR products obtained from the thermal cycler were digested with a lineage I enzyme (*NdeI*). This 10 µL restriction digest contained isolate PCR product, enzyme, buffer and nuclease free water, which was digested according to the supplier instructions. This enzyme would cut DNA from lineage I isolates into 390 and 341 base-pair fragments. The enzyme-digested PCR products were separated using agarose gel

electrophoresis and the results can be seen in Fig. 3. The 100 bp ladder, (lane 1, Fig. 3), was also loaded with DNA loading dye. This was visualised using the Bio-Rad Gel doc XR+ System (Bio-Rad, South Africa), which was combined with Image Lab Software version 5.2.1. The negative control, lane 2, is an undigested amplicon. The positive control, lane 3, is *L. monocytogenes* MEN09, which is a lineage I isolate (Fig. 3).

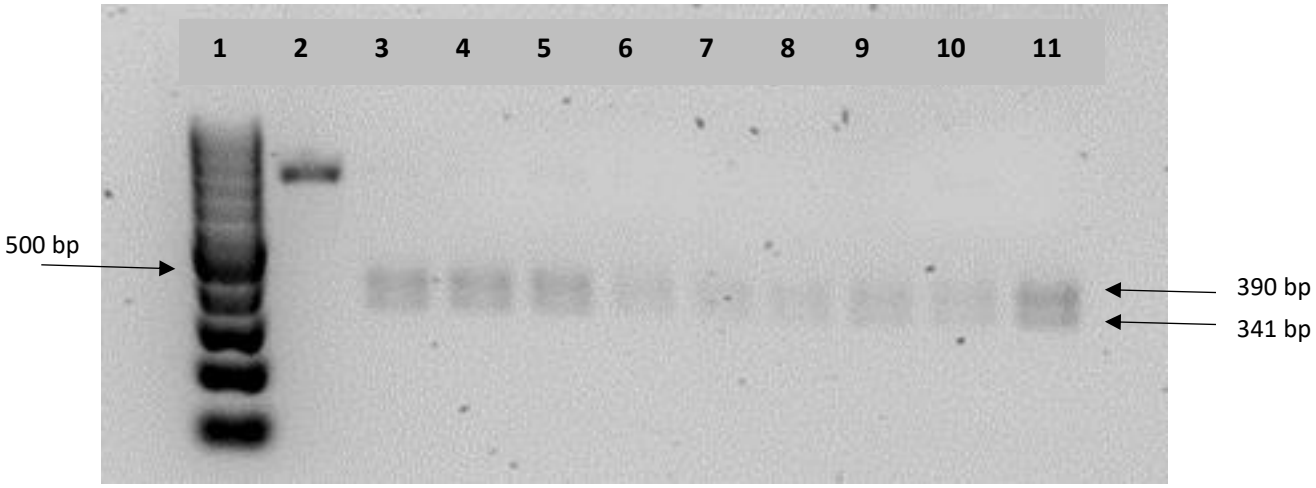

Figure 3 Lineage typing results of *L. monocytogenes* river water isolates, using the RFLP procedure, on a 2% agarose gel. Lane 1 = 100 bp ladder, Lane 2 = undigested amplicon, Lane 3 = *L. monocytogenes* MEN 09 (Lineage 1), Lane 4 = PR-01, Lane 5 = PR-02, Lane 6 = PR-03, Lane 7 = MR-01, Lane 8, MR-03, Lane 9 = FR-01, Lane 10 = FR-03, Lane 11 = FR-02

Dreyer *et al.* (2016) state that lineage I strains are most commonly reported in clinical infections and are linked to animals, whereas lineage II is associated with the environment as well as foodborne outbreaks. Lineages III and IV are isolated very rarely, but according to Dreyer *et al.* (2016), are associated predominantly with animals. Pirone-Davies *et al.* (2018) states that *L. monocytogenes* isolates from lineage I are more virulent, on average, than lineage II. Den Bakker *et al.* (2008) & Pirone-Davies *et al.* (2018) indicate that lineage I isolates exhibit more clonal characteristics than lineage II, which indicates that genetic traits required for fitness are under strong selection.

Based on the results of the lineage typing (Fig. 3), it was determined that all river water isolates were from lineage I. This was confirmed by the fact that the *NdeI* FastDigest enzyme (Thermo Scientific, South Africa) cut the PCR amplicons into band sizes of 390 and 341 base pairs. These findings correlated with the procedure set out by Rip & Gouws (2020). Kayode *et al.* (2019) performed a study which characterised listeriosis outbreaks in the Southern African region. This study gathered information regarding all studies performed in Southern Africa that included the detection of *L. monocytogenes* from different sources. Most notably, 11 reports of *L. monocytogenes* prevalence in wastewater and river water sources were included from various locations in South Africa. Kayode *et al.* (2019) state that the presence of *L.*

monocytogenes in water could be attributed to food-processing factory effluent, sewer discharge, vegetation and run-off from land or dump sites. This consistent presence of *L. monocytogenes* in river water all over country indicates that this pathogen is capable of surviving adverse environmental conditions and is resilient to treatment processes designed to disinfect it (Kayode *et al.,* 2019). A study performed on *L. monocytogenes* isolates from soil and surface water in Austria noted that 33.3% of isolates were associated with lineage I serotypes and 66.67% were associated with lineage II serotypes (Linke *et al.,* 2014). A study performed by Lyautey *et al.* (2007) reported that lineage I isolates dominated (61%) during the summer, whereas lineage II dominated (77%) during autumn in Canadian river water isolates. It is evident from these previously mentioned studies, that lineage I isolates are commonly found in river water, and the findings in the current study correlate with this.

Study B: *Extended Spectrum β-Lactamase and Antimicrobial Susceptibility Testing of isolates*

The reduction in effectiveness of antimicrobials in the clinical, industrial and environmental industries is an ever-increasing concern, which has affected the healthcare systems for both humans and animals (Shaikh *et al.,* 2015, Blaak *et al.,* 2015). The use of the words antimicrobials and antibiotics are reported as interchangeable terms in literature. The ability of microorganisms to adapt themselves to survive antimicrobials is determined by three main mechanisms; intrinsic resistance, the acquisition of antibiotic resistance genes through horizontal gene transfer and mutations (Zhang *et al.,* 2017). Increased usage as well as the misuse of these drugs are two reasons for the dramatic increases in the development of resistances in recent years (Shaikh *et al.,* 2015). The World Health Organization (WHO) (2014) has declared that antimicrobial resistant bacteria are emerging pollutants of water.

The isolates indicted in Table 1 for Study B were tested for antimicrobial resistance to a variety of antimicrobial agents (Tables 3 and 4). The procedures set out by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020) for ESBL testing and the Clinical & Laboratory Standards Institute (CLSI, 2016) for antimicrobial susceptibility testing were followed. The river water isolates listed in Table 1 have not been screened for antimicrobial resistance before. Due to experimental limitations and technical breakdowns in 2020, the MALDI-TOF strain confirmation tests could only be done much later than the antimicrobial analysis. The selection of isolates used in this section of work was thus based on the presumptive identification of the strains prior to testing. This was based on the typical growth on the respective selective agars. In the case of the *L. monocytogenes* isolates, the isolate selection was made based on PCR confirmation (Study A) and the growth on the fastidious Mueller-Hinton agar.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on all *Enterobacteriaceae* and *L. monocytogenes* isolates indicated in Table 1 for Study B. The breakpoint zone diameters for each antimicrobial tested are presented in Table 5 for *Enterobacteriaceae* and Table 6 for *L. monocytogenes.* Isolates were classified as resistant if the breakpoint zone diameter measured across the antimicrobial disc was smaller than the breakpoint diameter, and deemed susceptible to that particular antimicrobial if the zone diameter was larger than that suggested by EUCAST (2020). Positive and negative controls were included for both *Enterobacteriaceae* and *L. monocytogenes* isolates which were stipulated in the EUCAST procedures. These controls included *E. coli* ATCC 35218 (positive control), and *E. coli* 29522 (negative control) for *Enterobacteriaceae*. *Streptococcus pneumoniae* ATCC 49619 was included as the quality control for *L. monocytogenes* testing. All tests were performed twice, with duplicate plates prepared for each isolate. All results were averaged, and indicated in Tables 8 and 9 below. The results of MALDI-TOF analysis are indicated the respective columns in Tables 8 and 9. Isolates noted with a N/A in Tables 8 and 9 were not tested with MALDI-TOF analysis as these isolates are identification-confirmed reference strains. Figure 4 shows a MH-F plate which has been inoculated with *L. monocytogenes* following disc dispensing.

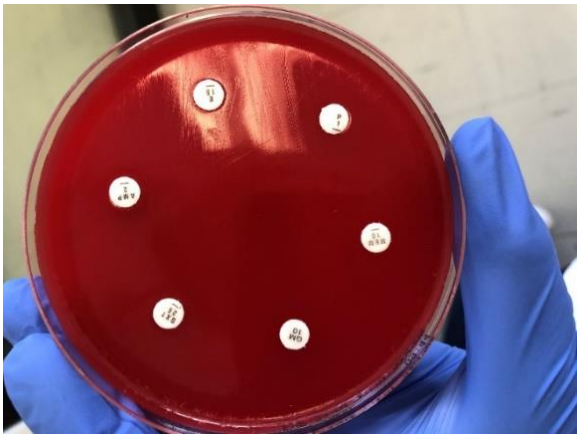

Figure 4 A Mueller-Hinton-F plate, with the antimicrobial discs dispensed, prior to incubation to test for *L. monocytogenes* antimicrobial resistance

Blaak *et al.* (2015) explains that the ingestion of microorganisms that have resistance to antimicrobials introduces both direct and indirect risks for humans. The direct risk is the introduction of infections that are incredibly difficult to treat. Indirectly, the ingestion of resistant microorganisms that are harmless to a healthy individual, are able to colonise the gut or skin even in healthy humans, and results in an asymptomatic carriage of that bacteria. This may result in gene transfer from the resistant bacteria to the naturally-occurring commensal

bacteria in the body, resulting in commensal bacteria carrying the resistance genes, which could cause opportunistic infections in immunocompromised individuals (Blaak *et al.,* 2015).

Table 8 Antimicrobial susceptibility testing results of *Enterobacteriaceae* isolates, and indication of the multidrug resistance (MDR) of the isolates to the antimicrobials tested

Bacteria	**Antimicrobial**									**MALDI-TOF ID confirmation**
	AMP	STX	GM	C	TE	CIP	NA	S	MDR Yes/No	
E. coli ATCC 35218	R	R	S	R	S	R	-	-	Yes	N/A
E. coli ATCC 29522	S	S	S	S	S	S	-	-	No	N/A
E. coli FR-01	R	R	S	S	R	S	-	-	Yes	Confirmed
E. coli FR-02	R	R	R	S	R	I	-	-	Yes	Confirmed
E. coli MR-01	R	R	S	S	R	R	-	-	Yes	Confirmed
E. coli BAA-2469	R	R	R	R	R	R	-	-	Yes	N/A
Salmonella MR-02	R	R	S	S	R	S	S	R	Yes	Confirmed
Salmonella PR-02	R	R	S	S	R	S	R	R	Yes	Confirmed
S. braenderup BAA-664	R	S	S	S	S	S	S	S	No	N/A
Salmonella - poultry	S	S	S	S	S	S	S	S	No	Confirmed

*MR – Mosselbank River, PR – Plankenburg River, FR – Franschhoek River **AMP – ampicillin, STX – trimethoprim sulfamethoxazole, GM – gentamycin, C – chloramphenicol, TE – tetracycline, CIP – ciprofloxacin, NA – nalidixic acid, S – streptomycin R – Resistant, S – Susceptible, I – Intermediate. N/A – not tested during MALDI-TOF analysis

Blaak *et al.* (2015) states that surface water is a major concern for the presence of antimicrobial resistant microorganisms mainly through the contamination of water by animal faeces, agricultural runoff, or through the discharge of improperly treated sewage wastewater into surrounding rivers and dams. Through recreational activities as well as the irrigation of crops with antimicrobial resistant microorganisms, humans may be exposed to this bacteria.

The results presented in Tables 8 and 9 provide an indication of the antimicrobial resistance profiles of the river water isolates (from Chapter 3) as well as the other ATCC, clinical and environmental strains that served as reference strains (Table 1). Alarmingly, all but one (*L. monocytogenes* FR-01) of the river water isolates (90%) could be classified as MDR based on the definition of showing resistance to at least one antimicrobial in three or more antimicrobial classes (Basak *et al.,* 2016). River water isolates tested for antimicrobial susceptibility testing included *E. coli* (n=3), *Salmonella* spp. (n=2) and *L. monocytogenes* (n=5). Variances were noted in resistance profiles of the isolates to certain drugs within the same river, such as the Mosselbank *L. monocytogenes* isolates, MR-01 and MR-03. The MR-01 isolate showed susceptibility to penicillin, whereas MR-03 showed resistance to this

antimicrobial (Table 9). This could indicate that there are different *L. monocytogenes* strains present in the river. All *Enterobacteriaceae* river water isolates were resistant to ampicillin, tetracycline and trimethoprim-sulfamethoxazole (Table 8). In Table 9, 80% of *L. monocytogenes* river water isolates (n=4) showed resistance to penicillin and erythromycin. Singer *et al.* (2016) states that the drivers of antimicrobial resistant bacteria in the environment are linked to domestic and industrial discharge of biocides and other chemicals, animal defecation, agricultural runoff and overuse of antimicrobials in both humans and animals. The prevalence of multidrug resistant microorganisms in river water was extremely high. This results in limited treatment options for individuals that may fall ill as a result of contamination from these microorganisms. In 2017, the World Health Organization developed a list that classified antimicrobials according to their importance for application in the medical industry. The antimicrobials used in the current study falls into either the critically important or highly important categories of this list (WHO, 2017). Therefore, the resistances reported to these extremely important antimicrobials further raises concerns.

Table 9 Antimicrobial susceptibility testing of *L. monocytogenes* isolates, and indication of the multidrug resistance (MDR) of the isolates to the antimicrobials tested

Strain/ Source	**Antimicrobial**								
	AMP	STX	GM	P	MEM	E	**MDR** Yes/No	**MALDI-TOF ID confirmation**	**PCR ID confirmation**
LM MR-01	R	R	R	S	S	R	Yes	Unconfirmed	Confirmed
LM MR-03	R	R	R	R	S	R	Yes	Confirmed	Confirmed
LM FR-01	S	R	S	R	S	S	No	Unconfirmed	Confirmed
LM PR-01	R	R	S	R	R	R	Yes	Unconfirmed	Confirmed
LM PR-03	R	R	S	R	S	R	Yes	Unconfirmed	Confirmed
LM MEN09	R	R	R	R	R	R	Yes	N/A	N/A
LM MEN32	R	R	R	R	R	R	Yes	N/A	N/A
LM ATCC 23074	R	R	R	R	R	R	Yes	N/A	N/A
S. pneumoniae ATCC 49619	R	R	R	R	S	R	Yes	N/A	-

*MR – Mosselbank River, PR – Plankenburg River, FR – Franschhoek River ** AMP – ampicillin, STX – trimethoprim sulfamethoxazole, GM – gentamycin, P – penicillin, MEM – meropenem, E – erythromycin R – Resistant, S – Susceptible, I – Intermediate N/A – not tested during MALDI-TOF analysis. LM – *Listeria monocytogenes*

A study performed by Blaak *et al.* (2015) on the presence of antimicrobial resistant bacteria in Dutch surface waters and wastewater indicated that 26% of surface water isolates were resistant to at least one antimicrobial. This was slightly lower than the presence of

antimicrobial resistant bacteria found in wastewater treatment plant effluent (31%). This study found that *E. coli* isolates were resistant to ampicillin, streptomycin, tetracycline and trimethoprim-sulfamethoxazole more often than to antimicrobials such as chloramphenicol, cefotaxime and ciprofloxacin. Sivhute (2019) analysed the antimicrobial resistance profiles of *E. coli* (n = 7) isolated from the Krom, Eerste and Plankenburg Rivers in the Western Cape. Two of these rivers were also included in the current study. Findings from Sivhute's study indicated that 100% of isolates were resistant to ampicillin, while 79% of the strains also showed resistance to tetracycline. In the current study, 100% of *Enterobacteriaceae* river water isolates, from the Franschhoek, Plankenburg and Mosselbank rivers (n=5), were resistant to tetracycline. All *Enterobacteriaceae* isolates (n=5) and 80% of the *L. monocytogenes* isolates (n=4) were resistant to ampicillin (Table 8 & 9). The *Enterobacteriaceae* resistance against ampicillin, therefore, correlates with Sivhute's (2019) *E. coli* resistance patterns, where two of the same rivers were analysed. Lamprecht *et al.* (2014) analysed the presence of MDR *E. coli* strains in the Plankenburg River over a period of three years. The highest frequency of resistance was reported against ampicillin and trimethoprim-sulfamethoxazole, followed by tetracycline. The fact that similar results are noted in the current study indicate that the use of these rivers without pre-treatment may pose a health risk for the consumers of fresh produce irrigated with this river water. In Table 1, it can be seen that *E. coli* BAA-2469 (NDM-positive) is known to be resistant to all antimicrobials except for nitrofurantoin and tigecycline (ATCC, 2019). The findings in the current study confirms that this isolate is resistant to all of the antimicrobials tested in this study.

A study performed by Akinyemi *et al.* (2011) on antimicrobial resistance profiles of *Salmonella* isolates from surface water sources in Nigeria showed that 80% of the 37 isolates tested were resistant to at least two of the antimicrobials tested. Eight of the thirteen antimicrobials used in this study by Akinyemi *et al.* (2011) were utilised in the current study. In the study performed by Akinyemi *et al.* (2011), resistance to ampicillin, tetracycline and streptomycin was reported most frequently (62.2%, 56.8%, and 75.7%, respectively). Resistance to trimethoprim-sulfamethoxazole was only reported in 8.1% of the isolates tested. The *Salmonella* isolates in the current study showed resistance to ampicillin, tetracycline, streptomycin and trimethoprim-sulfamethoxazole (Table 8). According to Montville *et al.* (2012d), *Salmonella* is not a stranger to surface waters, which are frequently used for irrigation without pre-treatment. It has also been proposed by Ryan & Andrews (2018) that 75% of *Salmonella* spp. strains isolated from African countries are MDR.

Another important factor to consider is the effect of UV radiation on antimicrobial resistance profiles in microorganisms. Alcántara-Díaz *et al.* (2004) states that UV radiation is able to completely inactivate microorganisms through DNA damage. If microorganisms are

not killed following UV radiation, mutagenesis to damaged DNA may be a result of improperly repaired DNA or the inability to remove damaged DNA. Alcántara-Díaz *et al.* (2004) found that *E. coli* isolates responded to physical and chemical DNA-damaging agents in a similar manner that bacteria would respond to environmental stresses through mutation generation and adaption. These microorganisms, with damaged DNA as a result of incomplete disinfection, may pass this damaged DNA onto daughter cells which may further increase the resistance profiles of microorganisms in the rivers.

Zhang *et al.* (2017) summarises that conflicting reports describe how UV radiation affects antimicrobial resistant bacteria. Munir *et al.* (2011) noted that there wasn't a significant reduction in antimicrobial resistant bacteria after UV radiation was applied at full-scale in a wastewater treatment plant. Whereas, Guo *et al.* (2013) and Huang *et al.* (2013) state that UV radiation is sufficient for the disinfection of antimicrobial resistant bacteria in water samples. The findings from the study performed by Guo *et al.* (2013) indicated an increase in antimicrobial resistant bacteria to certain antimicrobials after 20 $mJ.cm^{-2}$ of UV radiation was applied. Rizzo *et al.* (2014) found that ciprofloxacin resistance decreased in *E. coli* isolates after UV was applied and Pang *et al.* (2016) reported greater resistance against ampicillin after 40 $mJ.cm^{-2}$ of UV radiation was applied compared to the same isolates before UV radiation was applied. Zhang *et al.* (2017) states that there are limited studies on the role that genes conveying antimicrobial resistance play during UV radiation. These findings indicate that if UV radiation does not completely eradicate the MDR microorganisms from the water sources prior to irrigation, or if photoreactivation occurs, that the risk of microorganisms showing even greater resistance profiles may develop.

The findings from the current study highlights that antimicrobial resistance is prevalent in microorganisms found in river waters. The risk that these harmful microorganisms may be passed from irrigation water to fresh produce is a concern for consumer safety. In Chapter 3, it was determined that the Franschhoek River was the 'best case scenario' in terms of microbial and physico-chemical characteristics. In the current study, it can be seen that the isolates from this river exhibited similar antimicrobial resistance profiles as the isolates from the Mosselbank River, which was deemed to be the 'worst case scenario'. This indicates that even though the microbial loads may be lower in the Franschhoek River, the presence of MDR bacteria still poses a threat. The findings in this study correlate with literature that antimicrobial resistance of microorganisms is a cause for concern, especially with regard to fresh produce that may not be heat treated before consumption. These findings further exacerbate the need for effective water disinfection steps, to reduce the risk associated with the consumption of contaminated fresh produce.

Extended-spectrum β-Lactamase (ESBL) testing

Variation exists in literature with regard to the precise definition of an ESBL-producer (Paterson & Bonomo, 2005; Shaikh *et al.,* 2015). However, the most widely accepted description is an *Enterobacteriaceae* strain that produces the specific enzyme, β-lactamase, which is capable of conferring resistances to Penicillins and Cephalosporins (1st, 2nd and 3rd generation) as well as Aztreonams. Furthermore, ESBLs are inhibited by β-lactamase inhibitors such as clavulanic acid (Paterson & Bonomo, 2005). Table 10 indicates the isolates tested, as well as the results obtained in the duplicate tests per isolate to determine the presence of ESBL-producers. A microorganism is considered to be a Group 1 ESBL-producer if the inhibition zone diameter measured around the CTX or CAZ discs that are in combination with CV are ≥ 5 mm larger than those without CV. The isolate was considered to be a Group 2 ESBL-producer if the inhibition zone diameter around the CPM disc with CV was ≥ 5 mm larger than the CPM disc without CV added (Poulou *et al.,* 2014, Sivhute, 2019). A Group 2 ESBL producer includes *Enterobacteriaceae* that contain inducible chromosomal AmpC (Laubscher, 2019, EUCAST, 2020). Sivhute (2019) found that 11 of 14 (79%) *E. coli* isolates from the Krom, Eerste and Plankenburg Rivers were ESBL-producers. In contrast, the *Enterobacteriaceae* strains that were tested as part of this study did not yield similar results.

ESBL's can be separated into 8 main types. The SHV, TEM, CTX, OXA, PER, GES, BES-1 and VEB-1 types all exhibit variances in the functional properties of the β-lactamase enzyme (Shaik *et al.,* 2015). Shaik *et al.* (2015) states that the first-choice method of treating infections caused by ESBL-producing organisms is through the use of Carbapenems such as imipenem or meropenem, amongst others. However, resistance to these Carbapenems has emerged in recent years. Rawat & Nair (2010) states that ESBLs are encoded by the same plasmid that carries resistance genes for antimicrobial agents such as tetracycline, chloramphenicol and trimethoprim-sulfamethoxazole. Due to the fact that ESBL production is plasmid-mediated, an isolate may be able to contain ESBL-producing as well as non ESBL-producing cells within the same species (Yadav *et al.,* 2015). Teklu *et al.* (2019) states that because non ESBL-producing cells may be present even when ESBL-producing cells are present, resistance to antimicrobials across a broader spectrum than only β-lactams may occur. It was important to understand how the river water isolates in the current study were resistant to multiple antimicrobial agents but were not ESBL-producers.

Thomson (2010) indicates that this could be as a result of AmpC genes. Ye *et al.* (2017) states that ESBLs and AmpC-producers make up an important branch of the *Enterobacteriaceae.* The difference between ESBL-producers and AmpC-producers is summarised by Rodríguez-Baño *et al.* (2018). ESBL-producers are inhibited by β-lactam inhibitors and are incapable of hydrolysing Cephamycins, whereas AmpC-producers are not

inhibited by β-lactam inhibitors, confers resistance to Cephamycins and are incapable of effectively hydrolysing cefepime (Rodríguez-Baño *et al.,* 2018). ESBLs are typically encoded by plasmid genes, whereas AmpC genes can either be encoded for by plasmid genes or chromosomal genes (Rodríguez-Baño *et al.,* 2018). Chromosomally-mediated AmpC genes, which have commonly been located in Gram-negative bacilli, may result in the resistance to multiple antimicrobial agents such as Penicillins, Azetronam and Cephalosporins, therefore, the same as ESBL-producers. Multidrug resistances are often associated with plasmid-mediated AmpC β-lactamases in *Enterobacteriaceae* (Thomson, 2010). This is confirmed by Jacoby (2009), who states that plasmids that carry the AmpC β-lactamase genes are also able to carry other resistance genes, which would make that particular bacteria resistant to Aminoglycosides, chloramphenicol and tetracycline, amongst others. AmpC-producers are resistant to a broader range of antimicrobial agents than ESBL-producers (Tepeli & Zorba, 2018). The results (Table 10) show that none of the river water isolates tested were ESBL-producers.

Table 10 Extended-spectrum β-lactamase testing of all *Enterobacteriaceae* isolates

Zone diameter (mm)	CTX		CTX/CV		CAZ		CAZ/CV		CPM		CPM/CV		ESBL producer
Organism	1	2	1	2	1	2	1	2	1	2	1	2	Yes/ No
E. coli MR-01	11	14	11	11	11	16	15	20	17	27	19	33	No
E. coli FR-01	29	32	32	31	30	28	30	30	32	33	33	31	No
E. coli ATCC BAA-2469	11	11	11	14	11	12	13	11	14	12	15	13	No
E. coli ATCC 35218	33	28	33	32	30	28	30	29	32	31	30	32	No
E. coli ATCC 29522	32	33	31	28	28	25	29	28	31	32	32	32	No
Salmonella MR-02	31	31	34	33	28	26	29	30	31	30	31	33	No
Salmonella PR-02	29	31	31	31	30	26	33	30	31	26	32	31	No
Salmonella – poultry	30	31	28	32	24	25	27	28	28	29	32	31	No
S. braenderup ATCC BAA-664	31	32	32	33	25	12	29	25	32	27	32	34	No
K. pneumoniae 700603	18	24	34	31	13	12	26	25	26	27	30	30	Yes

*MR – Mosselbank River, PR – Plankenburg River, FR – Franschhoek River
** CTX – Cefotaxime, CAZ – Ceftazidime, CPM – Cefepime, CV – Clavulanic Acid

Correa-Martínez *et al.* (2019) state that AmpC-producers are less prevalent than ESBL-producers, however, have been identified in several outbreaks around the world. A great

concern with AmpC-producers is that they are often resistant to Carbapenems, which is the first-choice drug for ESBL-producers. Lan *et al.* (2017) states that there is currently no standardised method for the detection of AmpC-producers. Over-expression of the AmpC gene has commonly been reported in *E. coli.* This may result in bacteria testing negative for being ESBL-producers which can cause negative consequences for the treatment of patients (Lan *et al.,* 2017). Multiplex PCR is an effective method of determining if an isolate is an AmpC-producer (Lan *et al.,* 2017). A study was performed by Ye *et al.* (2017), on the presence of ESBL-producers and AmpC-producers in river water in China. A total of 43 *Enterobacteriaceae* isolates were obtained from rivers in eight different districts, where 11.6% and 16.3% of isolates were ESBL-producers and AmpC-producers, respectively (Ye *et al.,* 2017). Zurfluh *et al.* (2013) states that surface waters are at a particularly higher risk for harbouring resistance genes due to the fact that surface waters are the recipients of bacteria from a multitude of sources. More locally, a study performed in Gauteng Province in 2019 analysed the presence of ESBL-producers and AmpC-producers in fresh vegetables such as spinach, tomatoes and cucumber (Richter *et al.,* 2019). Supermarkets, farmer's market stalls and street vendors were used to obtain the 545 vegetable samples. Seventy-seven isolates were selected as they were presumptive ESBL-producers. Phenotypic analysis of these isolates confirmed that 79.2% of those selected isolates were ESBL-producers and 41.6% were AmpC-producers. This study concluded that these findings of the presence of multidrug resistant microorganisms as well as ESBL- and AmpC-producers in raw vegetables in Gauteng are the first documented for this region (Richter *et al.,* 2019). Due to the fact that PCR screening was not performed on the river water isolates in the current study, it cannot be confirmed that the presence of the AmpC gene may be inhibiting the positive test for ESBL, or if in fact, that the isolates do carry the AmpC gene at all. This means that, the organism may still show MDR while testing negative for ESBLs.

Study B was performed to determine the resistance profiles of the selected isolates, as well as to compare these resistances to other clinical, ATCC and environmental isolates of the same species of bacteria. It can be concluded that the river water isolates show MDR to the antimicrobials tested. These findings further highlight the requirement for effective treatment methods to ensure a consistently safe supply of water for irrigation. The ESBL test results indicate that none of the isolates were ESBL-producers. This does not rule out the possibility of ESBL presence in these rivers, as these organisms have been identified before in previous studies in the Krom, Plankenburg and Eerste Rivers. AmpC gene expression should be explored in future research as there is currently a gap in literature regarding expression in environmental *Enterobacteriaceae* isolates in South Africa.

Study C: *Comparison of resistance profiles of river water isolates to ATCC and enviromental strains to low-pressure UV radiation.*

Various strains of bacteria were exposed to selected doses of LP UV radiation, to determine the variations in resistance profiles between isolates obtained from different sources. The selection of river water isolates used in this study were limited to those who showed a high similarity to strains included in the MALDI-TOF database, resulting in positive strain confirmation results during MALDI-TOF analysis (Tables 8 and 9). This experiment also served to determine the efficacy of LP UV radiation against an increased microbial concentration in autoclaved river water. Table 1 includes a complete list of all bacterial isolates used in this experiment. The river water isolates were obtained during river profiling described in Chapter 3.

Each of the isolates listed in Table 1 for Study C, were exposed to three doses of UV radiation (20, 40, 60 mJ.cm^{-2}) and the microbial populations were determined before and after each respective dose. This procedure was conducted in triplicate for all strains. Figures 5 – 9 indicate the log cell counts before treatment (0 mJ.cm^{-2}) and after each of the doses. The results of the three rounds of testing are indicated in the following figures, where the colony forming units per mL have been averaged. The standard deviation (SD) (95% confidence level) error bars show the deviation in colony counts observed across duplicate plates poured, across six decimal dilutions, as well as the variation across the three testing occasions. Colony counts of between 10 – 300 colonies were recorded for *E. coli. Salmonella* spp. and *L. monocytogenes* were tested on a presence/absence basis only, and therefore, no colony were recorded.

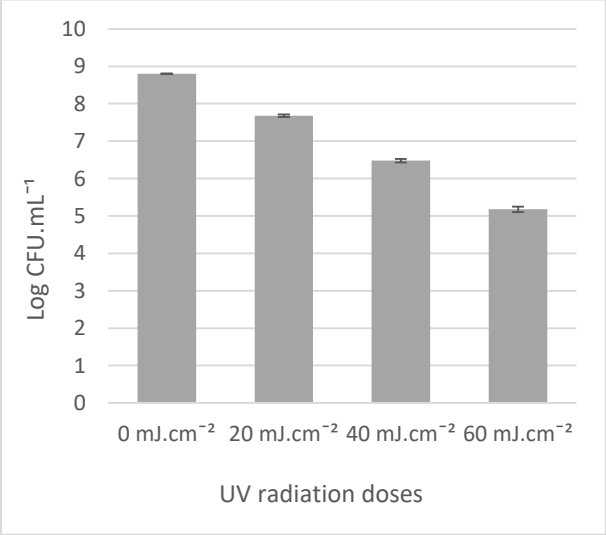

Figure 5 Response of the *E. coli* FR-01 isolate to varying doses of UV radiation, showing standard deviation error bars

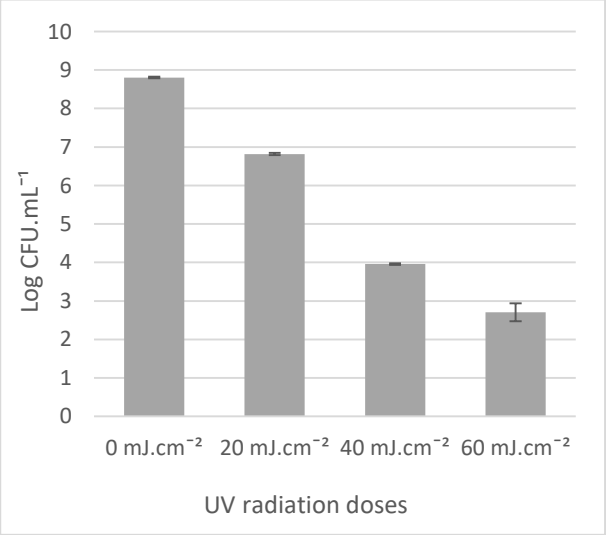

Figure 6 Response of the *E. coli* (CTX-TEM) to various UV radiation doses, showing standard deviation error bars

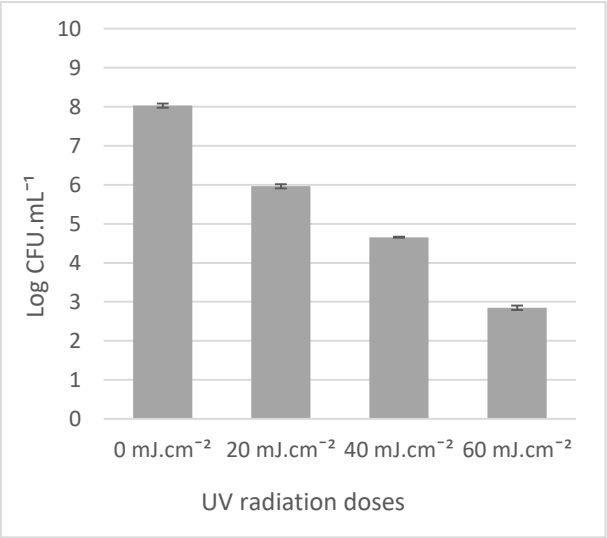

Figure 7 Response of the *E.coli* MR-01 isolate to the indicated doses of UV radiation, showing standard deviation error bars

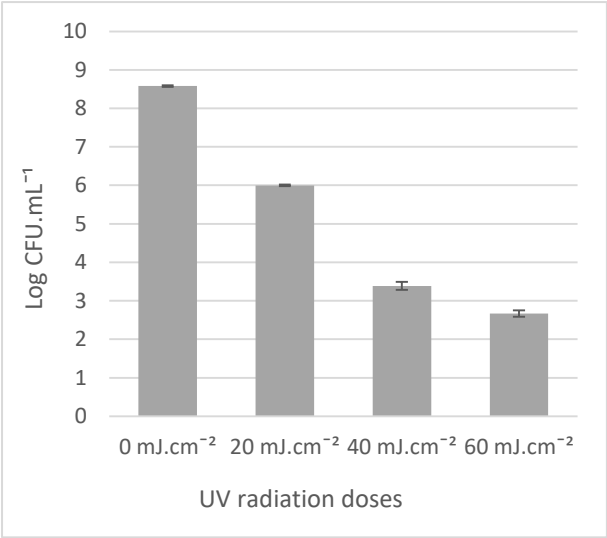

Figure 8 Response of *E.coli* strain ATCC BAA-2469 to the indicated doses of UV radiation, showing standard deviation error bars

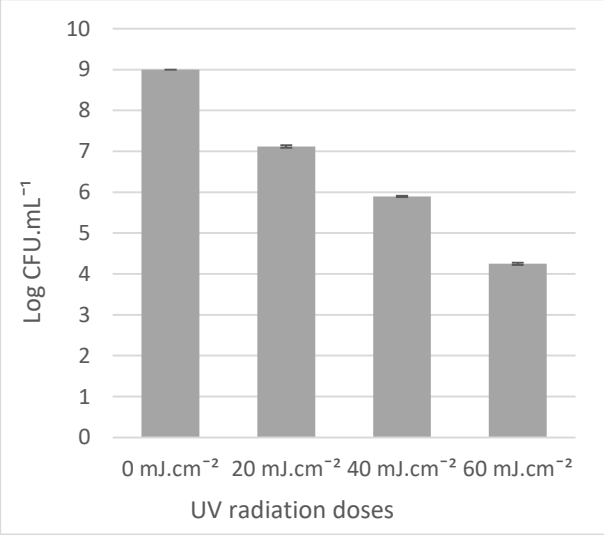

Figure 9 Response of the *E. coli strain* ATCC 35218 to the indicated doses of UV radiation, showing standard deviation error bars

Figures 5 – 9 indicate how the microbial loads present in the inoculums decrease as a result of UV radiation. The initial microbial concentrations (0 $mJ.cm^{-2}$) noted in this study, are much higher than those naturally observed in the river water. This enables the researcher to investigate the efficacy of this treatment method, even at high microbial concentrations (10^8 $cfu.mL^{-1}$). The initial microbial concentration was based on the method described by Zimmer and Slawson (2002) which was adapted for this study. Figure 5 and Figure 7 indicate the log reductions observed in the *E. coli* river water isolates, from the Franschhoek (FR-01) and

Mosselbank Rivers (MR-01), respectively. Figures 6, 8 and 9 are the *E. coli* isolates selected from alternative sources.

There is no significant difference between the initial microbial concentrations across all five isolates as well as all three sampling repetitions per strain ($p<0.05$). Any variation in results could, thus, primarily be linked to the intrinsic characteristics of the microorganism. This ensures the response of each strain to UV radiation can be compared directly, and speculations regarding resistances can be made. Gayán *et al.* (2014) explains that intrinsic parameters such as the cell size, cell wall thickness as well as the makeup of the genetic material of a microorganism may impact sensitivity towards UV radiation. It has also been reported by Hijnen *et al.* (2006) that microorganisms in their stationary phase show the greatest resistance to UV radiation, as compared to the growth phase.

In order to determine the strain that was the most resistant to UV radiation, the log counts of each strain after 60 $mJ.cm^{-2}$ were compared. The strain that had an average value of 5.2 log colony counts after the highest UV dose applied was the FR-01 isolate (Fig. 5). This was followed by the ATCC 35218 strain, with an average colony count of 4.2 log (Fig. 9). The Mosselbank River isolate was the third-most resistant strain, where an average of 2.8 log colony counts were reported after the highest dose applied (Fig.7). The BAA-2469 and CTX-TEM isolates closely followed with 2.7 log and 2.0 log colony counts each, respectively (Figs. 8 & 6). The reduction in the *E. coli* colony counts following each dose of UV radiation can be seen in Fig. 10.

The largest reduction in microbial load can be noted in the *E. coli* isolate (*E. coli* CTX-TEM), where an average reduction of 6.1 log was reported after 60 $mJ.cm^{-2}$ of UV radiation, followed by the *E. coli* BAA-2469 isolate with an average 5.9 log reduction after the same dosage (Fig. 10). The lowest log reduction noted, was found in the *E. coli* FR-01 isolate, where an average reduction of 1.1 log was reported after 20 $mJ.cm^{-2}$ of UV radiation was applied (Fig. 10). This isolate also had the highest microbial load present after the 60 $mJ.cm^{-2}$ of UV radiation (average of 5.2 log colony counts across the 3 repetitions) (Fig. 5). This is in comparison to a combined log reduction average of 3.1 for all four other isolates after 60 $mJ.cm^{-2}$ across the three testing repetitions (Figs. 6, 7, 8 & 9).

It is interesting to note, that although the Franschhoek River was considered as the best-case scenario for overall river quality based on the results presented in Chapter 3, these results indicate that the *E. coli* isolate obtained from this river (FR-01), is the most resistant to UV radiation of the stains tested. A study performed by Mofidi *et al.* (2002), found that laboratory-cultured *E. coli* strains (such as ATCC strain 23229) were more sensitive to UV radiation than pathogenic, clinical or environmental isolates (i.e. O157:H7). Hijnen *et al.* (2006)

state that environmental *Salmonella* and faecal coliforms were significantly more resistant than microorganisms of the same species that have been laboratory-cultured, this finding agrees with the findings in the current study.

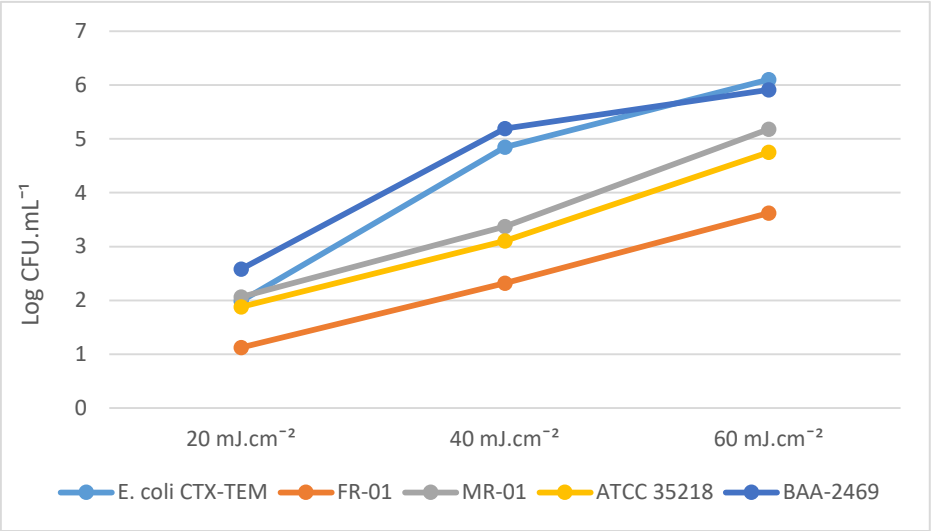

Figure 10 Residual log cfu. mL^{-1} of various strains of *E. coli* after three doses of UV radiation

Many conflicting reports exist in literature with regard to specific doses required for particular log reductions of *E. coli* strains. A report by Chevrefils *et al.* (2006) compiled a table of the suggested dose requirements that have been presented in literature. Contradiction exists in dose requirements for microorganisms. For example, an *E. coli* ATCC 11229 strain was reported by six different research papers to have seven different dose suggestions for a three-log reduction, ranging from < 3 – 9 $mJ.cm^{-2}$ (Chevrefils *et al.,* 2006). This variation provides a great deal of confusion for applying effective dosages for the required disinfection, and reflects the state of knowledge that exists. The choice of either LP or medium-pressure lamps can impact dosage requirements due to the variations in electrical output between the lamps, and should be taken into account prior to radiation (United States Environmental Protection Agency (USEPA), 2003).

Hijnen *et al.* (2006) states that mechanisms of DNA repair have evolved over time due to microorganism exposure to UV radiation from the sun. Therefore, environmental isolates may potentially have greater repair potential in comparison to isolates that have not previously been exposed to sunlight. Although DNA repair was not investigated during this study, it can be speculated that environmental strains possess certain intrinsic characteristics that distinguish them from reference strains due to the variation in stimulus provided by the environment (Hijnen *et al.,* 2006). These variations in intrinsic characteristics could result in increased resistances to disinfection treatments (Olivier, 2015). Bacteria that has previously

been exposed to starvation, osmotic stress, heat or acid has shown that co-protective adaptive responses can be triggered (Van der Veen & Abee, 2011).

Goldman & Travisano (2011) state that microorganisms that are frequently exposed to UV radiation (such as environmental strains that are exposed to sunlight), have greater repair mechanisms to reduce the UV damage to DNA. Their study reported on the exposure of *E. coli* isolates to repeated rounds of acute UV doses for a number of days, after which the changes in sensitivity or resistance profiles were recorded. A three-fold increase in resistance to UV radiation was reported in the strains subjected to repeated UV exposure, compared with duplicates of strains that were not previously exposed (Goldman & Travisano, 2011). These adaptive responses, to repair DNA damage as a result of UV radiation, often are responsible for dramatic alterations to other physiological traits within the cell. This results in greater variation in UV resistance within a strain. This evolution of microorganisms', towards an increase in UV resistance, could result in enhanced sensitivities to other stresses, such as osmotic, saline, chemical or antimicrobial. Goldman & Travisano (2011) state that the microorganisms that were consistently exposed to acute UV doses showed poorer survival during exposure to saline conditions.

The *E. coli* isolate containing the CTX-TEM gene has previously been identified as an ESBL-producer, which has shown resistance to penicillins as well as third generation Cephalosporins (Pfeifer *et al.,* 2010). A study performed by Zhang *et al.* (2017) stated that *E. coli* isolates that show multiple antibiotic resistances are more resistant to low doses of UV radiation, and require at least 20 $mJ.cm^{-2}$ of radiation for effective disinfection. It has been reported that antibiotic resistant microorganisms have the potential to survive UV radiation, as well as have better repair mechanisms than those that aren't resistant to antimicrobials (Zhang *et al.,* 2017). Following UV treatment of cefotaxime-resistant *E. coli,* a 0.5 log increase in bacterial counts was reported after a 3-day storage period (Silva *et al.* 2018). In this study, it was observed that the ATCC 35218 *E. coli* isolate was the second-most UV resistant isolate, considering the microbial counts observed after the highest dose of radiation applied (Fig. 8). This strain is known to be resistant to ampicillin, but has also shown resistance to antimicrobials such as Aminoglycoside and Macrolide drug classes (Romanis, 2013). *E. coli* ATCC 35218 is also a TEM-1 β-lactamase producer, which can confer resistance to Cephalosporins and penicillins (Salverda *et al.,* 2010, WHO, 2011, ATCC, 2020). Destiani & Templeton (2019) state that conflicting findings have been reported for the UV disinfection of antibiotic resistant bacteria. Guo *et al.* (2013) states that UV radiation results in an increased resistance to vancomycin, tetracycline and chloramphenicol, amongst others. A study performed by Destiani and Templeton (2019) reported that following UV radiation, antibiotic resistant *E. coli* strains were able to regrow and repair by up to 43% following a dosage of 10

mJ.cm^{-2}. Guo *et al.* (2013) states that this is could be due to the UV radiation resulting in the enrichment of bacterial resistance to antimicrobials. Therefore, it can be postulated that *E. coli* strains that show resistances to antimicrobials, might be less sensitive to UV radiation for disinfection.

Britz *et al.* (2012) and Britz *et al.* (2013) state that a target reduction of 3 – 4 log is suggested for water treatment strategies in order to ensure acceptable microbial loads in river water that is used for irrigation. This finding is based on the contamination levels that occur in local rivers in the Western Cape. A 3 – 4 log reduction target implies that if *E. coli* populations present in the river water range between 10^5 - 10^6 cfu. 100 mL^{-1}, the treatment would reduce the microbial population to a range that is considered safe according to the guideline stipulations (1 000 faecal coliforms per 100 mL) (DWAF, 1996). The initial microbial loads tested in this study, are much higher than that which would have naturally occurred in the river water. Based on the 3 – 4 log reduction target, it is clear that 20 mJ.cm^{-2} is not sufficient to ensure satisfactory disinfection. This finding implies that the higher the microbial load, the higher the dosage requirement for effective disinfection. Therefore, it is suggested that a dose of at least 40 mJ.cm^{-2} is required to achieve this target. A combined average log reduction of 3.6 was reported across all isolates after the application of 40 mJ.cm^{-2} (Figs. 5–9). However, this reduction in the artificially concentrated microbial inoculums prepared for this study was still not sufficient to reach levels below the guideline limit. This is as a result of the initial concentration being experimentally adapted to 10^8 cfu.mL^{-1}. When a microbial population as high as 10^8 cfu.mL^{-1} is reported, a 6-log reduction target is suggested. Therefore, an acceptable dose for this target is 60 mJ.cm^{-2} when the initial concentration is 10^8 cfu.mL^{-1}. In fact, the Mosselbank River isolate (Fig. 7) was the only isolate that had counts fall within the guideline stipulation after 60 mJ.cm^{-2}. Interestingly, again, the Mosselbank River was perceived as the 'worst-case scenario' of the selected rivers. Although the initial concentrations used in this study were considered to be unnaturally high, Sivhute (2019) reported *E. coli* counts of up to log 6.5 cfu.mL^{-1} tested in the Plankenburg River. Therefore, high concentrations have been reported before, occurring naturally in the tested rivers, which indicates a consistently high health risk.

Ultraviolet radiation has been successfully utilised in the disinfection of pathogens such as *Cryptosporidium and Giardia spp.,* as well as *L. monocytogenes* and *S. enterica* in water sources (Jones *et al.,* 2014). The pathogen isolates selected in this study (*Salmonella* spp. and *L. monocytogenes*) were exposed to the same treatment as the *E. coli* isolates. A variation in isolate sources was included to determine the environmental influence on UV resistance profiles. The results of the presence/absence testing of *Salmonella* spp. and *L. monocytogenes* isolates (all done in triplicate) can be seen in Table 11 and Table 12,

respectively. Due to experimental challenges, all *Listeria* strains could not be evaluated simultaneously. All experiments, except for *L. monocytogenes* ATCC 23074 and *L. monocytogenes* MR-03, evaluated in Study C were tested using the same batch of autoclaved river water. These two isolates were tested with a separate batch of Plankenburg River water. All physico-chemical characteristics of river water used are listed in Table 7. As the cultivation and experimental conditions were kept consistent during this study, extrinsic parameters such as the growing conditions, growth phase and recovery conditions would not significantly impact the treatment efficacy. A "+" sign in Tables 11 & 12 is indicative of a positive presence of that particular pathogen, and a "-" indicates that that pathogen was not present after testing.

Table 11 Presence or absence testing of *Salmonella* spp. isolates after various doses of UV radiation

Salmonella strain	MR-02 Isolate			PR-02 Isolate			*Salmonella* - Poultry source			*S. braenderup*		
Repetition / UV dose	1	2	3	1	2	3	1	2	3	1	2	3
0 mJ.cm^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
20 mJ.cm^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
40 mJ.cm^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
60 mJ.cm^{-2}	+	-	-	+	-	-	+	+	+	+	+	+

MR – Mosselbank River, PR – Plankenburg River

The isolates were treated in the same manner, with the absorbance values recorded prior to the application of UV radiation in order to determine the initial bacterial concentration, as the *E. coli* isolates. This ensures that the initial microbial concentration remains consistent across each repetition as well as between strains for direct comparisons to be made. Initial microbial loads were 10^8 cfu.mL^{-1} for all isolates. The results indicated in Table 11 & 12 provide an indication of the pathogen sensitivity to UV radiation. Gayán *et al.* (2011) states that Gram-negative microorganisms show greater sensitivity to UV radiation than Gram-positive microorganisms. This general rule of thumb, did not agree with the findings in this study. The *L. monocytogenes* (Gram-positive) river water isolate (MR-03) and the *Salmonella* spp. river water isolates (MR-02 & PR-02) (Gram-negative) both required at least 40 mJ.cm^{-2} of radiation for disinfection. In Table 12, Test 1 refers to the test with the first batch of sterile Plankenburg River, and Test 2 refers to the second batch. The physico-chemical characteristics of these two water samples can be seen in Table 7.

Table 12 Presence or absence testing of *L. monocytogenes* isolates after various doses of UV radiation

L. monocytogenes strain	MR-03			ATCC 23074 **Test 1**			ATCC 23074 **Test 2**			MEN09 (Lineage I)			MRA32 (Lineage II)		
Repetition / UV dose	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0 mJ.cm^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20 mJ.cm^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40 mJ.cm^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
60 mJ.cm^{-2}	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+

MR – Mosselbank River.
L. monocytogenes ATCC 23074 was tested twice, in two different samples of Plankenburg River water indicated by Test 1 and Test 2.

When comparing the resistance profiles of the *Salmonella* river water isolates (MR-02 & PR-02) to the *Salmonella* reference strains tested, it can be noted that the river water isolates showed greater sensitivity to UV radiation (Table 11). The prevalence of *Salmonella* spp. after the highest dose applied in the first testing repetition of the river water isolates could be attributed to slightly higher initial microbial concentrations in the first test compared to the second and third tests. In the first test, MR-02 had an absorbance reading of 0.24 in comparison to 0.198 and 0.211 on the second and third rounds, respectively. For the first testing repetition of the PR-02 isolate, an absorbance reading of 0.23 was recorded, in comparison to a reading of 0.197 and 0.207 in the second and third rounds, respectively. These isolates were obtained from the river water (Table 1) before UV radiation was applied, as the *Salmonella* spp. were completely inactivated after 20 mJ.cm^{-2} (Chapter 3). The prevalence of *Salmonella* spp. now, after 40 and 60 mJ.cm^{-2} shows that the increased microbial concentrations were too high for effective disinfection to occur. The highest dose (60 mJ.cm^{-2}) was also not sufficient for the disinfection of the high levels of *Salmonella* poultry isolate or the *Salmonella braenderup* isolate in the water, indicating that these organisms are more resistant to UV radiation than the river water isolates (Table 11).

Bucur *et al.* (2018) states that *L. monocytogenes* has shown greater resistance to UV radiation than other pathogens such as *E. coli.* Furthermore, other stresses such as osmotic stress, temperature or pressure do not impact or increase the UV resistance of this pathogen in any way (Bucur *et al.*, 2018). This is because the UV resistance of this organism does not depend on the stress sigma factor (SigB). The MR-03 isolate from the Mosselbank River was inactivated after a dose of 40 mJ.cm^{-2} of UV radiation was applied (Table 12). The dosage requirements for inactivation of the pathogenic microorganisms studied are relatively similar

across the selection of external isolates. For the river water isolates of *Salmonella* spp. and *L. monocytogenes,* the dosage requirements for inactivation is almost identical.

Zimmer-Thomas *et al.* (2007) state that DNA repair following UV radiation can occur via light dependent (photoreactivation) or light independent reactions. This study by Zimmer-Thomas *et al.* (2007) concluded that microbial repair following UV radiation is more pronounced at UV doses below 20 $mJ.cm^{-2}$, which was observed in *E. coli* O157:H7 isolates that were studied. Gayán *et al.* (2015) states that exposing microorganisms to sublethal environmental conditions may result in the triggering of adaptive responses that cause increases in the resistance profile of the microorganism. The study by Gayán *et al.* (2015) concluded that UV resistance is not dependent on the SigB factor, and that sublethal UV radiation did not increase resistance. Due to the fact that there was minimal growth on the agar plates after UV radiation in Chapter 3, no isolates were stored for testing in this section. A recommendation for future research would be to determine the resistance profile of isolates to UV radiation following survival after being exposed to UV radiation prior. When comparing the results obtained in Study B to the results shown in the current study, it can be seen that microorganisms that show resistance to antimicrobials, also show resistance to UV radiation.

The results of this experiment indicate that UV radiation is not sufficient to inactivate pathogens such as *Salmonella* spp. and *L. monocytogenes* at high concentrations, even at the highest dose applied. These concentrations (± 10^8 $cfu.mL^{-1}$) can be considered as unnaturally high concentrations that would not necessarily be found occurring in the environment. Based on the UV sensitivities of river water isolates, a dose of 60 $mJ.cm^{-2}$ for *Salmonella* spp. is advised, and for *L. monocytogenes* isolates, a recommended dose is 40 $mJ.cm^{-2}$. It is, however, recommended that more river water isolates be tested to provide a trend of the dosage requirements for inactivation. These findings also indicate that environmental isolates of these pathogens are not more resistant to UV radiation than the other non-environmental isolates. The limitation of testing for the presence/absence of an organism is that it is not known what the log reductions are, or how quickly they are reduced, making it difficult to make suggestions for the most economic treatment conditions.

Conclusions

In this study, the river water isolates obtained in Chapter 3 were characterised further. It was determined in Chapter 3 that the water quality of the rivers analysed were unsuitable for irrigation without pre-treatment. In the current study, the resistance profiles of the isolates were analysed and compared to other isolates from various sources.

In Study A, the lineage typing results of the *L. monocytogenes* river water isolates indicated that every test isolate came from lineage I. This lineage is the most frequent lineage associated with listeriosis infections and is reported to be more virulent than lineage II. According to literature, the source of lineage I *L. monocytogenes* strains has been associated with animals. This indicates that contamination of the river water with *L. monocytogenes* is possibly due to animal presence upstream.

In Study B, the antimicrobial resistance profiles as well as the presence of ESBL-producers were tested. It was determined that all but one of the river water isolates were MDR (90%), from the *Enterobacteriaceae* and *L. monocytogenes* analysis. The ESBL testing indicated that none of the river water isolates were ESBL-producers. These findings do not rule out the possibility of ESBL-producers in these rivers, as these organisms have been isolated before in previous studies in two of the same rivers. The negative test for ESBL-producers in the current study could be because that organism was missed on that sampling occasion, or the possibility of over-expression of the AmpC gene in the isolates resulted in a negative outcome for ESBL-producer test. This phenomenon has been reported in literature. However, it is recommended that future research investigates this possibility further, especially in environmental isolates. The multidrug resistance reported in the river water isolates is alarming. Treatment options for individuals or animals that fall ill as a result of these pathogenic organisms becomes limited by the amount of resistances developed against commonly used antimicrobials. The presence of antimicrobial resistant bacteria, as noted in the rivers in the current study show that even if microbial loads are low, there may still be a great risk involved with the transference of antimicrobial resistant bacteria in the water to fresh produce.

In Study C, it was determined that UV radiation was not sufficient to disinfect *E. coli* suspensions in river water when the microbial concentration is high (approximately 10^8 $cfu.mL^{-1}$), even at the highest dose applied. The concentrations of the microbial inoculums used in this study were higher than the microbial loads commonly reported in these rivers. It was determined that the Franschhoek River *E. coli* isolate exhibited the greatest resistance to UV radiation. This was an interesting finding as this river was deemed to be the 'best-case scenario' in Chapter 3, in terms of the microbial and physico-chemical profile of the river water.

The *L. monocytogenes* results from Study C indicated that the UV radiation was able to reduce microbial loads in river water isolates to below detectable levels after 40 $mJ.cm^{-2}$. This indicated that UV radiation was effective at disinfecting *L. monocytogenes* suspensions, even if the initial microbial concentration is elevated. The *Salmonella* spp. results of Study C indicated that approximately 40 $mJ.cm^{-2}$ was sufficient for disinfection. The presence of *Salmonella* spp. after 60 $mJ.cm^{-2}$ in the first repetition could be attributable to a slightly elevated initial microbial concentration on that testing round. For presence/absence testing, it is difficult to determine the rate at which the microbial concentration decreased after UV radiation.

In this chapter, it can be seen that the food pathogens investigated are potentially dangerous if ingested from contaminated fresh produce by consumers. The microorganisms that showed resistance to multiple antimicrobial agents in Study B (*Salmonella* MR-02 & PR-02, *L. monocytogenes* MR-03 and *E. coli* MR-01 & FR-01) also showed resistance to UV radiation in Study C. This provides an indication that microorganisms that are able to withstand one external stress, may show greater capabilities of resistance to others, such as antimicrobials. It is recommended that more river water isolates, that includes *Enterobacteriaceae* and *L. monocytogenes,* be tested to provide a trend of the resistance profiles over various seasons. *E. coli* river water isolates were not screened for pathogenicity, which is a recommendation for future research.

It can be concluded that the prevalence of these pathogenic microorganisms in the river water raises concern for the safety of users. Not only is this a risk for farmers using this water for irrigation purposes, but also for individuals that may use this water for other purposes. As previously discussed, the risk of microorganism carry-over from contaminated irrigation water to crop is a cause for concern, as many fresh produce-related disease outbreaks have been traced back to the irrigation water quality. This risk is heightened by the fact that minimally processed foods such as fresh produce are not heat treated prior to consumption. Specifically for *L. monocytogenes,* the ability to survive in a range of environmental conditions, further increases the concern for possible contamination.

The consistent presence of these pathogenic microorganisms in river water, throughout the testing period, as well as the high indicator loads reported in the same rivers prior to the current study, indicate that the use of these rivers is unsuitable for the irrigation of fresh produce without treatment. This could possibly be an indication of a constant contamination issue, from animal matter, human wastewater effluent as well as naturally occurring organisms. Based on the microbial resistance profiles of the river water isolates in this study, the need for an effective treatment method is confirmed. Ultraviolet radiation has successfully

been used at reducing the indicator and pathogenic microorganism present in irrigation water to safe or non-detectable levels.

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Chapter 5

INVESTIGATING THE EFFECT OF A BAG FILTER ON THE PHYSICO-CHEMICAL AND MICROBIAL PROFILE OF THE MOSSELBANK RIVER FOR IMPROVED UV DISINFECTION

Abstract

The effect of a bag filter was tested on water obtained from the Mosselbank River in an attempt to improve the overall characteristics of the water prior to disinfection with Ultraviolet (UV) radiation. Microbial and physico-chemical analyses were performed before filtration, and after using bag filters with four different pore sizes (5, 20, 50 and 100 µm). Microbial tests included *Escherichia coli (E. coli), Enterobacteriaceae* and Heterotrophic Plate Count detection and enumeration. The physico-chemical tests performed aided in the determination of the efficacy of the bag filter at removing suspended pollutants in the river water. Following filtration, low-pressure (LP) UV radiation was applied to the water samples. Microbial analyses, which included the aforementioned indicator tests with the addition of *Salmonella* species (spp.) and *Listeria monocytogenes* tests, were performed before and after UV radiation to determine the disinfection efficacy.

The bag filter results indicated a significant reduction ($p < 0.05$) in the physico-chemical results of the total suspended solids, turbidity, chemical oxygen demand and total dissolved solids content of the river water, after the smallest bag filter (5 µm) was used. The total dissolved solids content, even after bag filtration was still significantly higher than the guideline limit of 260 $mg.L^{-1}$. A greater increase in the Ultraviolet transmission percentage (UVT %) was expected through the reduction in total suspended solids, turbidity, total dissolved solids and chemical oxygen demand in the water.

The microbial loads in the river were not expected to be significantly reduced through the bag filter, however, notable reductions were reported in all indicator tests before filtration vs. after the smallest bag filter (5 µm). A 0.48, 0.43 and 0.4 log reduction was noted for *E. coli, Enterobacteriaceae* and Heterotrophic Plate Count colonies, respectively, from before treatment to after filtration with the 5 µm bag filter. These reductions in colony counts were not significant ($p > 0.05$), however, the microbial content of the water was still not acceptable for use following filtration, according to the guideline limits.

It was determined that the 5 µm bag filter provided the best results. This bag filter was selected for future experiments. Disinfection, through the application of LP UV radiation, was performed at three doses (20, 40 & 60 $mJ.cm^{-2}$). No *E. coli* nor *Enterobacteriaceae* colonies were reported after the lowest of UV (20 $mJ.cm^{-2}$) was applied. The Heterotrophic Plate Count colonies were lowered more gradually, which required 60 $mJ.cm^{-2}$ for complete disinfection.

Neither *L. monocytogenes* nor *Salmonella* spp. were present after the lowest dose of UV radiation was applied. The significant reduction in microbial loads, as well as absence of pathogen presence after the application of UV radiation, again, confirmed the efficacy of LP UV radiation as a method of river water disinfection.

Overall, the bag filter did improve the characteristics of the river water, but it was concluded that the extremely high total dissolved solids content of this river prevented a greater improvement in the UVT %. As a result, the UV exposure times were not impacted in this study through the use of a bag filter. The water was, however, deemed safe for use following the application of UV radiation, as it met the guidelines for microbial limits for the irrigation of fresh produce.

Introduction

Water quality is defined by the microbial, physical and chemical characteristics (Bhagwan, 2008). The physico-chemical characteristics are the non-microbiological parameters used to determine the acceptability of water for use. The Irrigation Water Guidelines (Department of Water Affairs and Forresty (DWAF), 1996a) provide an indication of the acceptable microbial and physico-chemical characteristics for use in agricultural irrigation. More recently, du Plessis *et al.* (2017) and the Water Research Commission developed a set of site-specific, risk-based guidelines for irrigation water quality that forms a Decision Support System. These guidelines do not contain specified limits for all of the parameters under investigation, and it was, therefore, decided that the DWAF (1996a) guidelines would be followed so as to have values to compare to as a standard reference. These characteristics, as well as others, were analysed in the four rivers (described in Chapter 3), to establish a comprehensive profile of the rivers. The Mosselbank River was deemed to be the worst-case scenario, of the four rivers, due to the high microbial loads and consistent pathogen presence. It also had the worst physico-chemical characteristics of the rivers tested. Even though this river had the worst profile, it was determined (based on the results presented in Chapter 3) that Ultraviolet (UV) radiation was effective at reducing the microbial loads present in this river to acceptable levels, in spite of the poor physico-chemical characteristics. When using low-pressure (LP) UV radiation at laboratory-scale, the factor that greatly determines the exposure time required to apply a specific dose, is the Ultraviolet transmission percentage (UVT %). This parameter is affected by various factors in water, which include the total suspended solids (TSS), total dissolved solids (TDS), turbidity, chemical oxygen demand (COD) and sample depth (Hassen *et al.,* 2000). The TSS and turbidity of a water sample have an inverse relationship with the UVT %, according to a study performed by Farrell *et al.* (2018).

In an attempt to reduce the exposure times required to apply a specific dose of UV radiation, a pre-treatment step was implemented to improve the physico-chemical characteristics of the river water. Bag filters with four different pore sizes were selected for use in this experiment, where the effect on flow rate, microbial load and physico-chemical characteristics were analysed on three separate sampling occasions. Bag filters are intended to remove the physical pollutants present in a water sample (Kesari *et al.* 2011), however, the efficacy of the filtration is governed by the pore sizes of the filter (Okpara *et al.,* 2011).

Koutchma (2009) states that the application of UV radiation has become an attractive method of water disinfection due to the efficacy of microorganism disinfection. UV radiation is also environmentally friendly in comparison to other methods, such as chemical disinfection (Hijnen *et al.,* 2006). In Chapter 3, it was determined that LP UV radiation at laboratory-scale was effective at reducing the microbial loads to an acceptable level, at the doses investigated. Medium-pressure (MP) lamps are preferred in pilot-scale applications due to the high power output generated and increased intensity of the UV lamps, in comparison to LP UV lamps (United States Environmental Protection Agency (USEPA), 2003). This, therefore, results in a greater environmental footprint in comparison to MP UV (USEPA, 2003).

In this section of work, the first step towards the future transition between laboratory-scale LP UV and a MP pilot-plant is made by introducing the bag filtration step. With only one factor changing from the LP UV protocol followed in Chapter 3, the effect of filtration on the profile of the river water can be determined. The aim was to select the most effective bag filter for future application in a pilot plant-based research project that will follow on this Masters' project (as part of WRC project K5/2965//4.) Comparisons between the microbial loads that were identified during sampling in the dry, summer season and wet, rainy winter season could provide an indication of the effect of the environmental conditions on the microbial loads present.

The aim of the research presented in this study was to investigate, firstly, the effect of bag filtration on the microbial and physico-chemical profiles of the river water. Secondly, to determine the effect of LP UV radiation, at the same three doses as previously analysed (Chapter 3), on the microbial loads present following filtration. Physico-chemical and microbial analyses were performed before and after bag filtration at each different pore size. Microbial tests included the quantification of *Escherichia coli (E. coli), Enterobacteriaceae* and Heterotrophic Plate Count (HPC) loads. Following UV radiation, the testing for the presence of *L. monocytogenes* and *Salmonella* spp. was included, along with the indicator organism tests. Where possible, the microbial and physico-chemical characteristics were compared to the guideline limits for agricultural irrigation to determine acceptability for use (DWAF, 1996a).

Materials and Methods

In this chapter, the materials and methods section is divided into two parts. The first being the general materials and methods used. This is followed by the research design of the chapter.

General materials and methods

Sampling method

The Mosselbank River in Kraaifontein (-33.819729, 18.703042) is situated downstream from a sewerage treatment plant and is the source of water for large-scale commercial farmers. One thousand litres of water was obtained from this river in a tank, using a Honda L20xH petrol pump (Honda, South Africa) and transported back to the Department of Food Science for testing. The water was then run through four different bag filter pore sizes (5, 20, 50 & 100 µm) (Darlly, South Africa). Water samples were taken for microbial and physico-chemical analyses before treatment, and then after each different bag filter was used.

All microbiological tests were performed within six hours of sampling, and all physico-chemical tests were performed within 24 hours of sampling (American Public Health Association (APHA), 2005).

Physico-chemical analysis of river water samples

The physico-chemical characteristics of the river water were analysed before treatment and then after each bag filter was used. The results of these analyses were compared to the guideline limits obtained from the Irrigation Water Guidelines (DWAF, 1996a). These guideline limits can be observed in Table 1 of Chapter 3. All physico-chemical tests were performed in duplicate and average values were obtained.

Total Dissolved Solids

A (TDS)-3 meter (HM Digital) was used to determine the total dissolved solids (TDS) content of the river water. The meter gives a reading that is expressed as parts per million (ppm) which equates to $mg.L^{-1}$.

Turbidity

A portable Orion AQ3010 Turbidity Meter was used to determine the turbidity of the water (Thermo Scientific, USA). The turbidity is expressed in Nephelometric Turbidity Units (NTU). The instrument was calibrated prior to use, following the protocol from the supplier.

Alkalinity

A titration was performed, according to Standard Methods (APHA, 2005), to determine the alkalinity of the water samples. Fifty mL of water was placed in a beaker and titrated with a solution of 0.1 N H_2SO_4 until a pH reading of 4.3 was reached. The volume of H_2SO_4 required

to reach this pH was recorded which is used to determine the alkalinity in units of mg.L $CaCO_3^{-1}$.

Ultraviolet Transmission percentage

The Ultraviolet Transmission percentage (UVT %) of the river water was determined using a Sense T254 UV Transmission (%) Photometer (Berson, Netherlands). The instrument was calibrated using distilled water, as per protocol instructions.

Chemical Oxygen Demand

The Spectroquant Nova 60 Chemical Oxygen Demand (COD) cell test was used to determine the COD of the water samples (Merck Millipore, South Africa). Three mL of river water was pipetted into a vial containing the test reagents and digested for 2 hours at 148°C in a thermal reactor (Spectroquant TR 420) (Merck Millipore, South Africa). The COD values were obtained by using the Spectroquant Nova 60 Spectrophotometer (Merck Millipore, South Africa) and expressed in units of mg $O_2.L^{-1}$. The vials used from Merck Millipore (South Africa) are for samples that have COD readings in the range of 10 – 150 mg $O_2.L^{-1}$. There is no specified limit for COD in the Irrigation Water Guidelines (DWAF, 1996a), so the guidelines for water used for industrial-use was consulted instead (DWAF, 1996b).

pH

The pH of the river water was measured using a portable pH meter (WTW, Germany). The meter was calibrated first using standard pH solutions, according to the instructions provided by the supplier.

Total Suspended Solids

The APHA (2005) standard methods were consulted for the determination of the Total Suspended Solids (TSS) content. An amount of river water was filtered through a Munktell glass fibre 0.6 µm filter paper (Lasec, South Africa). The filter paper was dried in crucible at 105°C for 2 hours. The weights of the crucible and filter paper were recorded before filtration, and then after heating. The respective calculation was performed to determine the TSS value which is expressed in units of $mg.L^{-1}$.

Electrical conductivity

A portable HI 8733 conductivity meter was used to determine the electrical conductivity (EC) of the water samples (Hanna Instruments, USA). The EC of the water samples is expressed as $mS.m^{-1}$. The instrument was calibrated prior to use.

Microbial analysis of river water samples

Following sampling and bag filtration, microbial analysis was performed to determine the microbial profile of the river water by enumerating the following; *E. coli, Enterobacteriaceae* and HPC. Microbial enumeration and pathogen detection of *L. monocytogenes* and

Salmonella spp. were also performed following disinfection with UV radiation. Enumeration methods included a dilution series (10^{-1}-10^{-6}) that was prepared according to the SANS method 5221 and SANS method 6887-1 (SANS, 2011a, SANS, 1999). All methods were conducted in duplicate, and are discussed in the following sections.

Escherichia coli *identification and enumeration*

Brilliance Coliform/ *E. coli* Selective Agar was used to determine the presence and number of *E. coli* colonies in the water samples (Oxoid, South Africa). The instructions from (ISO Method 16654:2001) were followed. A presumptive positive test for *E. coli* was considered in the presence of purple colonies on this agar following incubation. The colonies in the range of 10 – 300, per plate, were counted and recorded.

Enterobacteriaceae *identification and enumeration*

The presence and enumeration of *Enterobacteriaceae* was determined by using Violet Red Bile Glucose Agar (Oxoid, South Africa), according to the procedure set out in the International Standards Organisation (ISO) Method 21528-2 (ISO, 2017). Pink coloured colonies were counted and recorded in the range of 10 – 300 per plate, following incubation.

Heterotrophic Plate Count enumeration

The Heterotrophic Plate Count (HPC) of the water samples was determined using Plate Count Agar (Oxoid, South Africa), as prescribed in the SANS method 4833-1 (SANS, 2007). Following incubation at 30°C for 72 hours, straw coloured colonies were enumerated and recorded when colony counts were in the range of 10 – 300 per plate.

Salmonella *species detection*

Salmonella spp. were identified by following the SANS method 19250 (SANS, 2011). Before and after each dose of UV radiation, a 25 mL water sample was inoculated into 225 mL sterile buffered peptone water (Oxoid, South Africa) and incubated at 30°C for 24 hours. Then, 0.1 mL of this incubated suspension was transferred to 10 mL sterile Rappaport Vassiliadis (RV) broth (Oxoid, South Africa) and incubated. Two selective agars, Xylose Lysine Deoxycholate Agar (XLD) and Hektoen Enteric Agar were prepared according to supplier instructions (Oxoid, South Africa). The streak plate technique was used for this analysis, and plates were incubated. A presumptive positive of *Salmonella* spp. was considered to be the presence of red colonies with black centre on the XLD agar, and blue-green coloured colonies with or without black centres on the Hektoen Enteric Agar.

Listeria monocytogenes detection

The presence of *L. monocytogenes* was determined by following the procedure set out in the ISO method 11290-1 (2017). A 25 mL water sample, before and after each dose of UV radiation, was inoculated into 225 mL sterile half Fraser broth together with a *Listeria* Selective

Supplement (BioRad, South Africa) and incubated at 30°C for 24 hours. Rapid'*L.mono* Agar Plates were streaked following the enrichment incubation (BioRad, South Africa). A presumptive positive result for *L. monocytogenes* was considered to be the presence of blue/black coloured colonies on the red agar plates, following incubation at 37°C for 24 hours.

Collimated beam procedure

The collimated beam system was utilised for UV radiation of water samples (Berson, Netherlands). This emits a beam of light from a mercury vapour lamp at 253.7 nm. A simplified diagram of this instrument can be seen in Figure 1. The LP mercury vapour lamp had an output power of 40 W and an arc length of 25 cm (UV-Technik, Germany). Prior to UV radiation, the light intensity of the UV lamp is determined at the surface of the sample, which is measured using an ILT1400 radiometer (International Light Technologies, USA) which is coupled with a XRL140T254 detector (International Light Technologies, USA).

The light intensity, sample depth and UVT % are used in the following equations to determine how long each sample needs to be exposed to UV light to receive a specific dose (Hallmich & Gehr, 2010).

$$I_{\text{avg},\lambda\,(mW.cm^{-2})} = I_0\lambda \left[\frac{1-e^{d\ln(\text{UVT}(\lambda))}}{-d\ln(\text{UVT}(\lambda))}\right] \quad [1]$$

Desired dose ($mJ.cm^{-2}$) = Average intensity ($mW.cm^{-2}$) *x* Exposure time (s) [2]

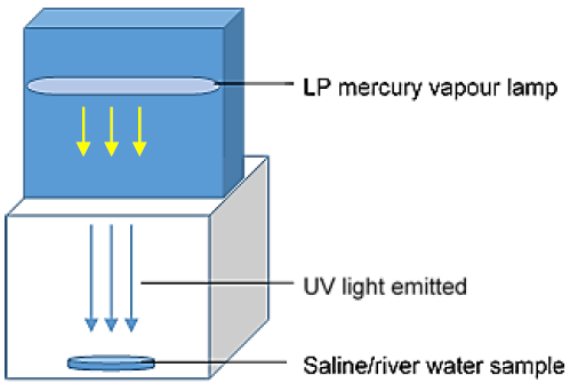

Figure 1 Bench-scale collimated beam device used to perform the laboratory-scale UV experiments (Berson, Netherlands) (Olivier, 2015)

Bag filtration procedure

Four bag filters of varying pore sizes were used in this study (5, 20, 50 & 100 µm). The bag filter fits into the pilot-scale plant. A sample was collected in a sterile container, before filtration, and then water was allowed to run through the bag filters for a set amount of time before samples were taken after filtration through each bag filter. These five samples (one before and four bag filter samples after filtration) were then analysed for the physico-chemical characteristics and indicator organism microbial loads.

UV treatment procedure

The application of UV radiation was performed in the same manner as the method reported in Chapter 3. The UV lamp was allowed to heat up for 10 minutes prior to the UV light intensity measurement being recorded. The UVT % of the water samples were then recorded and the relevant calculations were performed to determine the exposure times required for each dose of UV. The amount of water required for one treatment (dose) for all microbial tests is 51 mL. An amount of 25 mL was required each for *Salmonella* spp. and *Listeria monocytogenes* testing, and 1 mL was required for the dilution series for all indicator analysis. The amount of water for the 'before' treatment test, is removed from the water sample. As there are three UV doses, a measured sample of water (153 mL for all three doses) was placed in a sterile beaker. This beaker was placed on a magnetic stirrer, on a medium speed, in the collimated beam UV cabinet for the allocated time for the first dose (20 mJ.cm^{-2}). The 51 mL aliquot was then removed, in a sterile manner, and the beaker was returned to the cabinet for dose two (40 mJ.cm $^{-2}$) and dose three (60 mJ.cm $^{-2}$), repeating the same process. As the amount of water in the beaker decreases with each dose, so the time required to apply a dose decreases too. This sample depth reduction was taken into account in the equation used to calculate exposure times. Full microbial analysis, including pathogen testing, was then performed for the 'before' treatment test, and after each dose of UV radiation was applied.

Research Design

The aim of this study was to attempt to improve the physico-chemical characteristics of river water, prior to UV treatment, through the use of a bag filter, in order to increase the UVT % and enhance disinfection efficacy. The experimental design of this study was structured around two main objectives. Firstly, the effect of four different bag filter pore sizes (5, 20, 50 & 100 µm) on the physico-chemical and microbial profile from water samples of the Mosselbank River was determined over three sampling occasions. Secondly, the effect of UV radiation at three different doses (20, 40 & 60 mJ.cm^{-2}) on the microbial populations was determined following bag filtration. This river was selected as it was determined to be the

'worst-case scenario' irrigation source of the analysed rivers in Chapter 3 based on the microbial loads, consistent pathogen presence and poor physico-chemical water quality.

On each sampling occasion, water samples were obtained before (n=1) and then after each bag filter was used (n=4). These five samples were tested for their physico-chemical characteristics and indicator organism enumeration was performed. The results obtained in these experiments were compared to the Irrigation Water Guidelines (DWAF, 1996a), to determine the acceptability for agricultural irrigation.

Results and Discussion

Objective 1: *The effect of a bag filter on the physico-chemical and microbial profiles of the Mosselbank River*

Kesari *et al.* (2011) states that filtration methods are utilised for separating solid particles from liquid. This is the oldest method of water treatment. Okpara *et al.* (2011) indicates this method of treatment is limited by the pore sizes of the filter. In this section of the study, the effect of a pre-treatment step was determined through monitoring the changes in the physico-chemical characteristics and microbial loads. Four different bag filters were used, which had varying pore sizes (5, 20, 50 and 100 µm). The Mosselbank River was sampled on three separate occasions and the physico-chemical characteristics and indicator organism loads were determined before and after each different bag filter. The results of the physico-chemical analysis are presented in Table 1. The microbial analysis results are presented in Figures 2, 3 & 4. Results were averaged across the three sampling occasions.

Table 1 Physico-chemical results of river water before treatment and after bag filters of varying pore sizes, with standard deviation showing variation across the three sampling occasions for each bag filter

Characteristic	**Bag filter pore size**					
	Before	**100 µm**	**50 µm**	**20 µm**	**5 µm**	**Guideline**
UVT %	27.80	28.50	28.80	29.23	29.63	N/A
SD	*11.28*	*11*	*10.7*	*11.04*	*10.6*	
TDS ($mg.L^{-1}$)	728.67	727.17	725.23	722.67	715.33	260
SD	*25.54*	*24.5*	*23.43*	*24.46*	*25.58*	
EC ($mS.m^{-1}$)	0.85	0.86	0.84	0.84	0.83	40
SD	*0.02*	*0.01*	*0.03*	*0.03*	*0.03*	
COD (mg $O_2.L^{-1}$)	60.50	55.83	56.50	54.17	44.50	75
SD	*30.2*	*24.5*	*31.05*	*27.4*	*21.5*	
Turbidity (NTU)	28.68	24.78	24.80	23.55	22.68	10
SD	*1.55*	*2.43*	*1.55*	*1.06*	*2.3*	
TSS ($mg.L^{-1}$)	42.39	33.81	31.45	30.58	28.67	50
SD	*9.06*	*13.07*	*14.9*	*19*	*13.13*	
pH	7.36	7.36	7.37	7.36	7.34	6.5-8.4
Alkalinity (mg.L $CaCO_3^{-1}$)	153.33	161.67	158.33	163.33	158.3	120
SD	*30.1*	*30.14*	*31.12*	*25.7*	*33.3*	

SD – standard deviation, N/A – not available

The four bag filter pore sizes (5, 20, 50 & 100 µm) were selected in an attempt to not extend treatment time significantly by reducing the flow rate of the water through the system. If the flow rate of the water through the bag filter is slow, this will increase the time required for treatment application. From an economic feasibility standpoint, the most effective and efficient treatment will be favoured.

Hassen *et al.* (2000) and Gayán *et al.* (2012) indicate that the factors that have the greatest effect on the efficiency of this treatment method are the suspended solids, COD, and turbidity and UVT %. These are the interlinking factors that affect the transmission of the beam of UV light through a water sample. The pH, alkalinity and EC values were not expected to change significantly through the use of a bag filter. This is due to the fact that these parameters are mostly influenced by the dissolved solids content. According to Broséus *et al.* (2009), common methods of lowering the TDS content in water include reverse osmosis and ion exchange. Traditional bag filters are not commonly employed to lower the TDS content as the pore sizes are too large and there is no electrochemical attraction between the particles and the filter.

The results presented in Table 1 show how the use of a bag filter impacts the physico-chemical characteristics of the river water. The total dissolved solids content of the river water correlate with similar findings in Chapter 3, where extremely high values were noted on all sampling occasions. The Irrigation Water Guidelines (DWAF, 1996a) indicate that an acceptable TDS value is 260 $mg.L^{-1}$. The values obtained in this study, even after filtration, dramatically exceeded this guideline limit. A reduction in the TDS content from 728.67 to 715.33 $mg.L^{-1}$ was noted from before treatment to after the bag filter with the smallest pore size was used. There was a significant difference between the TDS content before and after filtration (5 µm) ($p < 0.05$). It was not expected that there would be a significant reduction in the TDS content. This is because the pore sizes of the bag filters are too large to physically remove dissolved solids. The small reduction in TDS content could be attributed to dissolved solids being bound or attached to the suspended solids that got filtered out. The dissolved solids content may be too small to be removed by filtration, however, they still impact the turbidity and ultimately the UVT % of a water sample (Fondriest Environmental, 2014).

The sampling point of this river was situated downstream from a wastewater treatment plant, which could have contributed to the high TDS content reported in this river. The dissolved solids could possibly be from the upstream activities, industrial effluent as well as the reagents used in the treatment plant for adjusting the water pH prior to releasing the water into the river. Üstün (2009) states that even though wastewater treatment plants are intended to remove pollutants from the water, a large portion of the dissolved metal content in the water remains or is even increased in a water treatment plant. Iloms *et al.* (2020) state that toxic

heavy metals such as Hg, Cd, Ar and Pb are persistent in wastewater treatment systems due to the fact that they aren't biodegradable as well as being recalcitrant in nature. The World Health Organisation (WHO) (2004) & Patoczka (2007) suggests that sodium hydroxide and carbonates are commonly used for pH adjustment after water treatment. Patoczka (2007) performed a study on the effect of various chemicals on the TDS content in water samples. The addition of sodium bicarbonates for neutralisation (pH > 8.3) increased the TDS level by 0.476 per $mg.L^{-1}$ of chemical added. Lime ($Ca(OH)_2$) is also a commonly used neutraliser in water treatment, which increased the TDS content by 1.35 per $mg.L^{-1}$ added (Patoczka, 2007). These chemicals also affect the alkalinity of the water following treatment, where the addition bicarbonates and hydroxides is positively correlated with an increase in alkalinity. Patoczka's study continued with an investigation into wastewater treatment plants which measured the change in TDS content following treatment. Overall, increases in TDS were reported across the treatment plants from influent to effluent (Patoczka, 2007). Patoczka (2007) concluded that the addition of chemicals will typically cause an increase in TDS content, and that chemical softeners will cause significant impact on the wastewater TDS content. These factors, therefore, may be responsible for the high TDS content present in the Mosselbank River water in the current study.

The measurement of the TSS content was performed by filtering the water through a 0.6 µm glass fibre filter paper. According to the American Public Health Association (APHA, 2005) standard methods, the total suspended solids present in a water sample is the amount of inorganic and organic material retained by a filter with pore size less than 2 µm. The TSS plays a major role in the scattering of light, according to Farrell *et al.* (2018), which has a negative impact on the UVT %. Suspended solids present in the water may shield microorganisms from UV radiation or absorb the radiation, resulting in ineffectively treated water (Abdul-Halim & Davey, 2016). In this study, there was a significant reduction in the TSS levels in the river water through the use of a bag filter ($p < 0.05$), which was expected. In contrast to the small 1.83% improvement observed for the TDS content, there was a 32.4% improvement in TSS following the use of the 5 µm bag filter. In a river with a lower TDS content, and a similar TSS content the Mosselbank River, the effect of the bag filter on the UVT % just through the removal of the suspended solids could potentially be much greater than observed in this study (Table 1). According to the guideline limits, the TSS content before and after filtration are acceptable for irrigation as they are less than 50 $mg.L^{-1}$ (Table 1) (DWAF, 1996a).

The COD values obtained before and after filtration show a 26.4 % decrease after the smallest filter (5 µm) was used. The COD value provides an indication of the level of organic pollution present in a water sample, expressed as the amount of oxygen required to oxidise all organic matter to carbon dioxide and water (Wu *et al.*, 2011). A reduction in COD was noted

from 60.5 mg $O_2.L^{-1}$ before bag filtration to 44.5 mg $O_2.L^{-1}$ after the 5 µm bag filter was used, which was significant ($p < 0.05$). This provides an indication of the interlinking relationship between the COD and TSS content in water. Hohenblum *et al.* (2017) investigated the possibility of a correlation between the COD and TSS content of water from wastewater treatment plants. A general relationship was established that the COD value tends to be 1.5 times the total suspended solids value. When applying this ratio to the results in Table 1, a similar ratio existed in the river water, which could indicate that the wastewater treatment plant effluent had a noticeable impact on the river profile. Hohenblum *et al.* (2017) further indicates that there is a greater relationship between the COD and suspended solids in a water sample than between the COD and turbidity. This is due to the fact that the COD reading does not include inorganic solids present in the suspended solids. According to the guideline limits for industrial use, which specifies irrigation water having a limit of < 75 mg $O_2.L^{-1}$ (DWAF, 1996b), this COD value of this river water is acceptable.

The turbidity of a water sample provides an indication of how much a beam of light would be scattered due to suspended particles (Hohenblum *et al.,* 2017). Butler & Ford (2018) indicate that there is a positive relationship between the TSS and turbidity of a water sample. Rügner *et al.* (2013) states that the relationship between the turbidity and TSS content is, however, dependent on a number of factors which includes the size, density, type and shape of the suspended solids present in the water. Therefore, relationships between factors are dependent on the upstream activities which are site-specific for each river. Both the TSS and the turbidity are known to have an inverse relationship with the UVT % (Farrell *et al.*, 2018). A reduction in the turbidity from 28.68 to 22.68 NTU was reported from before filtration to after the smallest bag filter (5 µm). It was reported that an increase in turbidity from 1 – 10 NTU could reduce the applied dosage by up to 33 % (Liu, 2005). The turbidity exceeded the guideline limit for domestic use (DWAF, 1996c) of < 10 NTU on all testing occasions.

The expectation was that the UVT % would be improved by a significant amount through the removal of suspended particles causing the scattering of light. In Table 1, a 1.83 % increase in UVT % was reported from before filtration, to after the smallest pore size (5 µm) was used. According to the USEPA (2003), an acceptable UVT % for effective disinfection in practice would be 75%. Based on the UVT % readings observed in the current study, an average UVT % of 28.8% is unacceptable. This result indicated that even though the COD, suspended solids and turbidity values were significantly reduced through the bag filter, the high TDS content present was responsible for affecting the UVT %. A study performed by Shahawy *et al.* (2018) indicated in principal component analysis experiments on the relationships between physico-chemical characteristics in river water; that TDS, TSS and the volatile suspended solids are positively correlated and these three components all correlate

negatively with the UVT %. Therefore, even though the parameters such as the TSS, turbidity and COD are decreasing with the pre-treatment step, the initial extremely high TDS content was still not reduced enough to have a positive impact the UVT %.

The experiments performed in this study were performed during June and July 2020, which is the rainy season in the Western Cape, South Africa. The establishment of the microbial and physico-chemical profile was performed in the dry, summer season (Chapter 3). When comparing the physico-chemical results between these two seasons for this river, the effect of the change in season and weather on the physical water quality can be determined. Parameters such as the TSS and turbidity increased in the wet season, but TDS and alkalinity decreased compared to the dry season. The UVT %, COD, pH and EC remained relatively similar during both seasons. These findings further highlight the interlinking relationship between the physico-chemical characteristics of the river water, where factors such as TSS and turbidity may increase in one season or the TDS in another season and the UVT % stays relatively consistent.

In terms of application, the lifespan of a bag filter is entirely site-specific and depends on the physical quality of the water that requires filtration (Fileder, 2020). The benefit of using this type of filter as a pre-treatment step is that, once full of suspended solids, it can be removed and cleaned before being returned and re-used. A decreasing flow rate is an indication that the bag filter needs to be cleaned (Fileder, 2020). The cost of a bag filter at the time of application in the current study (2020), was R353, 05 (Hans van Kamp, 2021, East Midlands Water Company, personal communication, 25 February). This, compared to membrane or sand filtration, is a more affordable pre-treatment step.

Overall, the bag filter did improve some of the physico-chemical characteristics of the river water. The expectation that the UVT % would be significantly improved was not met, which could be attributed to the extremely high TDS content in this river. The 5 µm bag filter was the most effective of the four sizes tested, at improving the physico-chemical characteristics of the water and did not reduce the flow rate of the water. This bag filter pore size was selected for pilot-plant experiments.

Microbial indicator levels present before and after each bag filter were determined and are represented in Figures 2, 3 & 4 as log cfu.mL^{-1}. Indicator organism tests included *E. coli, Enterobacteriaceae* and HPC. These tests were performed in duplicate for each dilution (10^{-1} – 10^{-6}) and an average reading was obtained. Plates were recorded with colony numbers between 10 and 300 colonies. Figure 2, 3 & 4 provide the results averaged across three sampling occasions.

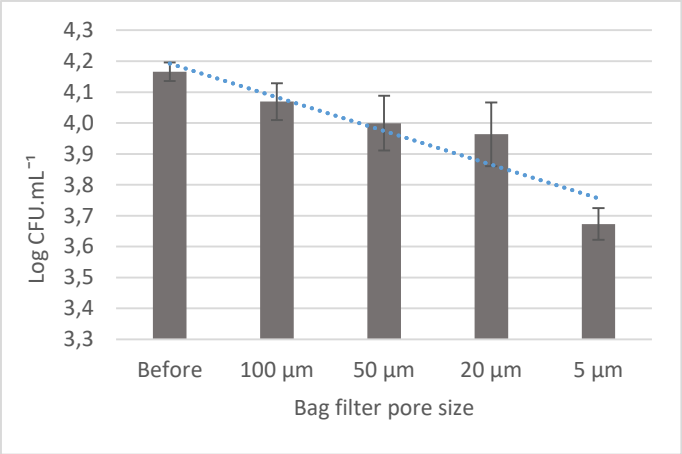

Figure 2 Average *E. coli* colony counts expressed as cfu.mL^{-1} before and after various bag filters were used, with standard deviation error bars indicating deviation across sampling occasions

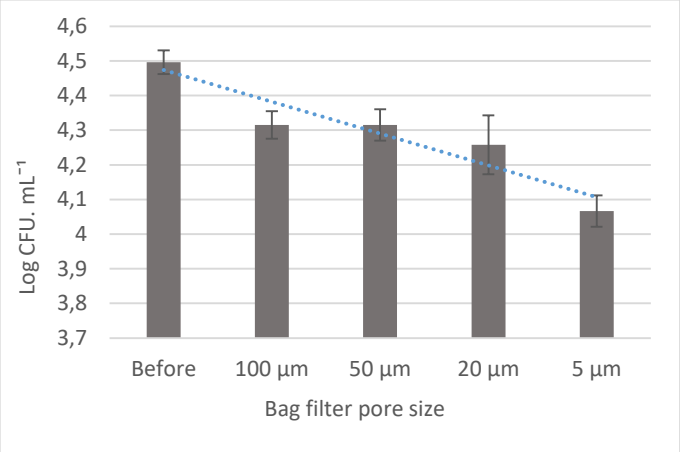

Figure 3 Average *Enterobacteriaceae* colony counts expressed as cfu.mL^{-1} before and after various bag filters were used, with standard deviation error bars indicating deviation across sampling occasions

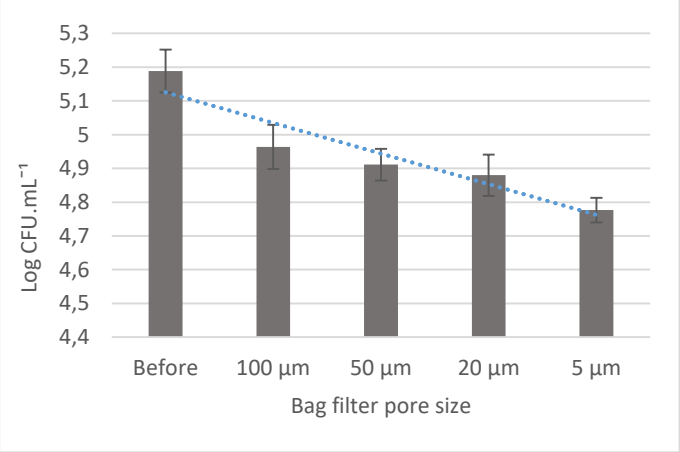

Figure 4 Average Heterotrophic Plate Count colony counts expressed as cfu.mL^{-1} before and after various bag filters were used, with standard deviation error bars indicating deviation across sampling occasions

Huey *et al.* (2010) performed a study on the correlation between physico-chemical characteristics and the microbial content present in various surface waters. It was reported that there was a positive correlation (R^2 = 0.747 and R^2 = 0.711) between the turbidity and TSS vs. the *E. coli* counts, respectively. It was concluded that these physico-chemical parameters are linked to the microbial loads present (Huey *et al.,* 2010).

In this study, the microbial populations were not expected to be significantly reduced through the use of a bag filter. This is due to the size of a bacteria being smaller than the pore sizes of the bag filters. An *E. coli* cell is approximately 1 – 2 µm in length and 0.5 µm in diameter (El-Hajj & Newman, 2015). Any reductions in microbial counts could possibly be attributed to bacteria being bound or attached to the suspended solids that got filtered out with the bag filter. Christensen *et al.* (2001) indicates that suspended solids present in water provide a medium for both the accumulation as well as transportation of bacteria.

A study performed by Berger *et al.* (1996) investigated how bacteria are able to colonise the suspended solids present in the Danube River, Austria. It was established that between 3 and 17 bacteria were able to colonise one suspended particle. This number is reportedly underestimated by up to 45% due to bacteria inside each particle being difficult to see under a microscope. It was also established that 39% of the suspended particles in this river were colonised by bacteria (Berger *et al.,* 1996). Therefore, it is highly probable that the reduction in colony numbers observed in this study were as a result of the bacteria being attached to the suspended solids that were removed through filtration.

Colony counts were recorded before and after each pore size to determine if the bag filter was able to remove any bacteria. Figures 2, 3 & 4 provide the results for the indicator organisms tested. In Fig. 2, the *E. coli* results indicate a 0.43 log reduction from before filtration to after the 5 µm bag filter. The reduction in colony counts after filtration were not sufficient for this river water to be considered safe for use, according to the guideline limits (DWAF, 1996a). This reduction in *E. coli* colonies was not statistically significant (p = 0.113). The *Enterobacteriaceae* counts were reduced too, by 0.475 log counts (Fig. 3). There are no specified limits for *Enterobacteriaceae* in the guidelines (DWAF, 1996a). There was not a significant reduction in the *Enterobacteriaceae* counts after filtration through the 5 µm bag filter (p = 0.379). When considering the *E. coli* log reductions following the three larger bag filters (100, 50 & 20 µm), a reduction of 0.10, 0.17 and 0.20 log cfu.mL^{-1} were reported, respectively. Similarly, *Enterobacteriaceae* log reductions that were noted following the three larger bag filters included 0.18, 0.18 and 0.24 log cfu.mL^{-1} reductions were reported, respectively. Heterotrophic Plate Count log reductions following the use of the three larger bag filters resulted in a 0.23, 0.28 and 0.3 reduction in log cfu.mL^{-1}, respectively. A higher initial microbial

load was reported for the HPC test (Fig. 4). The HPC test provides an indication of the total microbial population present in a sample, so these results were expected. A reduction in colony counts of 0.4 log were reported from before bag filtration to after the smallest filter was used. There are no specified limits for the HPC in the guidelines (DWAF, 1996a). There was not a significant reduction in the HPC after filtration through the 5 µm bag filter ($p = 0.316$). For all indicator organism tests, the reductions were not significant where average p-values across the three sampling occasions were 0.146, 0.245 and 0.282 for *E. coli, Enterobacteriaceae* and HPC, respectively.

The current study was performed in the winter, rainy season of the Western Cape. The initial profiling of this river was performed in the dry, summer season (Chapter 3). When comparing the indicator microbial loads between the two seasons, the impact of the environmental conditions on the microbial loads can be noted. An average *E. coli* count of log 4.64 cfu.mL^{-1} was recorded across the five sampling occasions in the summer, whereas an average of 4.15 cfu.mL^{-1} was noted for the three sampling occasions in the winter season. *Enterobacteriaceae* results show an average of 4.72 log cfu.mL^{-1} in the summer vs. 4.50 log cfu.mL^{-1} in the winter. Heterotrophic Plate Count (HPC) results indicate an average of 4.87 log cfu.mL^{-1} in the summer and an average of 5.17 cfu.mL^{-1} in the winter season. These results remained relatively similar over the two seasons and provide an indication of consistent contamination of this river. This is inspite of the water being heavily diluted in the wet, winter season. In a study by Tornevi *et al.* (2014), the effect of rainfall on the microbial populations present in a Swedish river was investigated. It was reported that precipitation events of more than 15 mm in 24 hours resulted in a three-fold increase in *E. coli* colonies. This finding did not correlate with the findings in the current study.

It is evident that the bag filter was able to remove some of the microorganisms present in the water sample. Even though this was not the intended aim of this experiment, it is a welcomed finding. Any reduction in microbial counts, even prior to treatment with UV radiation, aids in the pursuit of safe water. The 5 µm bag filter produced the best results with regards to reductions in microbial counts and physico-chemical improvement.

***Objective 2**: Results of microorganism response to UV radiation after bag filtration*

Three doses of UV radiation (20, 40 & 60 mJ.cm^{-2}) were applied to samples of water after filtration through the 5 µm bag filter. For the indicator organism analysis, plates were poured in duplicate and an average value was obtained. The results for indicator organism tests can be seen in Fig. 5, where the red line indicates the lowest detectable limit. Pathogen testing was performed on a presence/absence basis, and results for these experiments are indicated in Table 2.

In Fig. 5, it can be seen that, on all three sampling occasions, there were no *E. coli* or *Enterobacteriaceae* colonies recorded after the first dose of UV was applied (20 mJ.cm^{-2}). The results also indicate no growth after the second (40 mJ.cm^{-2}) and third (60 mJ.cm^{-2}) doses were applied. These microorganisms therefore, are sensitive to disinfection with UV radiation. Based on the initial microbial loads present over the three sampling occasions, average reductions of 3.5 and 3.15 log cfu.mL^{-1} were observed for *Enterobacteriaceae* and *E. coli* counts, respectively. The lowest dose (20 mJ.cm^{-2}) was required to reduce microbial numbers of these microorganisms to below detectable limits for the three doses applied (Fig. 5). A more gradual reduction in the HPC colonies was reported after UV radiation. Similar results were reported in the profiling of this river (Chapter 3). An average reduction in colonies for each dose of UV radiation was calculated as 2.34, 3.72 and 4.07 log cfu.mL^{-1} for 20, 40 & 60 mJ.cm^{-2}, respectively (Fig. 5). These findings are in agreement with a study performed by Sharrer *et al.* (2005) who investigated the effect of various doses of UV radiation on the microbial populations present. Heterotrophic Plate Counts and total coliform bacteria were analysed. In their study, a dose of 18 mJ.cm^{-2} was applied to a sample of water, which inactivated 100% of coliform bacteria present. The same dose applied, resulted in only a 98% reduction in the HPC of this water, which was a 1.7 log reduction (Sharrer *et al.,* 2005). Higher initial HPC microbial loads were reported in the current study in comparison to the study performed by Sharrer *et al.* (2005). Higher doses of UV radiation were also applied in the current study than this study. After 20 mJ.cm^{-2} an average reduction of 2.6 log cfu.mL^{-1} was recorded. This provides an indication of bacteria more resistant to UV radiation than *Enterobacteriaceae* being present in the microbial population, in the current study. Mofidi *et al.* (2002) performed a study on the levels of microbial survival and regrowth following high doses of UV radiation in water samples, specifically looking at *E. coli* and HPC counts. Following a dose of 140 mJ.cm^{-2} and then in dark storage at 20°C for 7 days, the HPC returned to the level it was prior to UV radiation. The *E. coli* results showed a 1-2 log increase in cfu.mL^{-1} following 24 hours of dark storage following a dose of 5 mJ.cm^{-2} (Mofidi *et al.,* 2002).

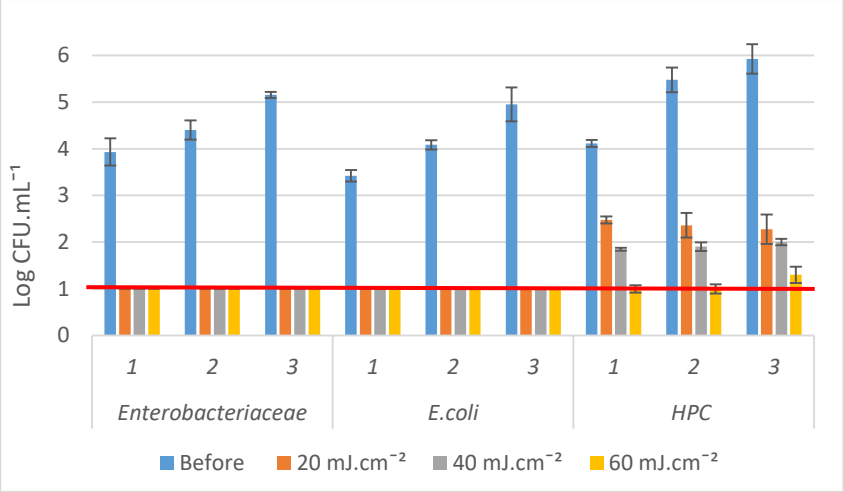

Figure 5 Indicator organism test results on three separate sampling occasions, after UV radiation was applied at three doses, with standard deviation error bars included for variation between duplicate plates poured, across six decimal dilutions. The red line indicates the lowest detectable limit of microbial presence

Although there was a more gradual reduction in the HPC colonies, there was only one sampling occasion where colonies were present after 60 mJ.cm^{-2} of UV radiation. No *E. coli* or *Enterobacteriaceae* colonies were present after the lowest dose of UV (20 mJ.cm^{-2}) was applied. This indicates that the UV radiation was effective at reducing the microbial loads present to an acceptable or safe level. Microbial recovery following UV radiation was, however, not investigated in this study. Since the *E. coli* and *Enterobacteriaceae* levels observed in the Mosselbank river appear to be constant, considering the results observed in this chapter and in Chapter 3, it is proposed that microbial recovery post UV be investigated in future.

The current study was performed in the wet, winter season. The profiling of this river was performed between the hot, dry, summer season and the wet, rainy season (Chapter 3). When comparing the effect of UV radiation on the microbial populations present in this river, conclusions can be drawn regarding the resistance profiles of microorganisms in different environmental conditions. This was of interest, specifically for the HPC colonies, as these organisms show a greater resistance to UV radiation. Figure 6 provides an indication of the HPC colony counts after UV radiation from summer to winter. In the winter analyses, the bag filter was included in treatment, which should be taken into consideration.

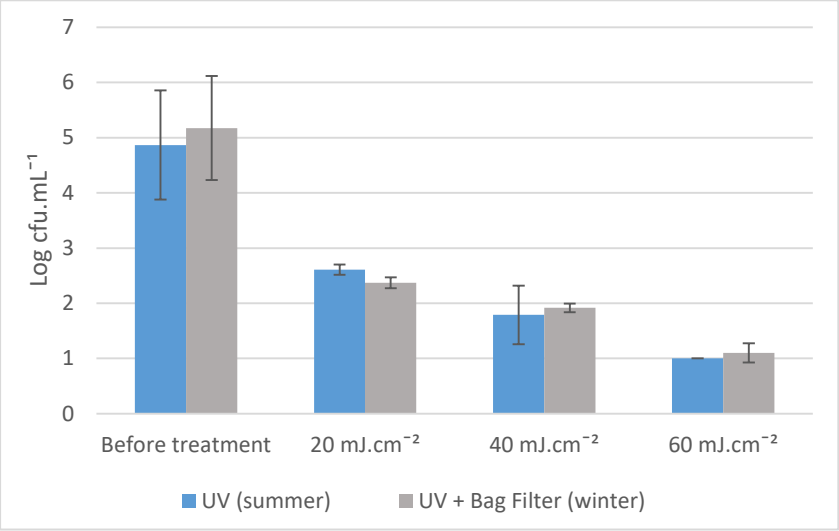

Figure 6 Comparison of HPC loads in the summer season versus the winter season, indicated as log cfu.mL^{-1}, with standard deviation error bars included

The results in Fig. 6 show that the effect of the environmental conditions on the microbial loads present in this river water was not significant (p = 0.953). However, when taking the reduction in colony numbers through the use of the bag filter (average of 0.4 log cfu.mL^{-1}), there are slightly higher microbial loads present in the winter season. The pathogens, *L. monocytogenes* and *Salmonella* spp. were tested before and after filtration, as well as after each dose of UV radiation. The results of these tests can be seen in Table 2.

Table 2 Presence absence testing results of pathogen before and after filtration, as well as after each dose of UV radiation

	Salmonella spp.			*L. monocytogenes*		
Treatment	1	2	3	1	2	3
Before	+	+	+	+	+	+
5 µm filter	+	+	+	+	+	+
20 mJ.cm^{-2}	-	-	-	-	-	-
40 mJ.cm^{-2}	-	-	-	-	-	-
60 mJ.cm^{-2}	-	-	-	-	-	-

No colony counts are reported, as this test was performed on a presence/absence basis only, therefore it is not possible to determine if there were any reductions in colony numbers due to the bag filter. Consistent pathogen presence was reported on all sampling occasions of this river (Table 2). The efficacy of UV radiation was successful, as neither pathogen was present after the first dose of UV was applied. This agrees with findings in Chapter 3, where no

pathogens could be detected after the lowest dose of UV (20 mJ.cm^{-2}) was applied. Yaun *et al.* (2003) indicate that 14 mJ.cm^{-2} of LP UV radiation is sufficient to cause a 5-log reduction in *Salmonella* spp. Similarly, a dosage requirement of 16.9 mJ.cm^{-2} resulted in a 5-log inactivation of *L. monocytogenes,* according to Matak *et al.* (2005). The results in the current study confirms the dosage requirements from these previously mentioned studies that a dosage of 20 mJ.cm^{-2} is required to inactivate the pathogens under investigation.

The consistent presence of pathogens in this river confirms that it is unacceptable for agricultural irrigation without treatment. The 5 µm bag filter was not expected to remove the pathogens, as the pore sizes of the bag filter are larger than the size of a bacteria, however the results indicate that some of the HPC, *E. coli* and *Enterobacteriaceae* cfu's were removed. However, UV radiation was effective at removing these pathogens, and lowering indicator organism loads to an acceptable level. This water would be deemed microbial safe, according to the Irrigation Water Guidelines (DWAF, 1996a) for agricultural irrigation.

Conclusions

In this study, the effect of a bag filter on the microbial and physico-chemical profile of the Mosselbank River were analysed. It was determined, through the significant reductions in the physico-chemical characteristics, that the 5 µm pore size bag filter provided the greatest results without reducing the flow rate of the water. The bag filter was effective at reducing the COD, TSS and turbidity levels in the water samples. Greater increases in the UVT % were expected through the use of the bag filter, however, the river has an exceptionally high TDS content, which could be preventing an improvement in UVT %. The bag filter did reduce the TDS content very slightly, but this was still not enough for the level to be considered acceptable according to the guideline limit of 260 mg.L^{-1}.

Microorganism reduction wasn't expected through the use of a bag filter, as even the smallest bag filter has pores larger the size of a bacteria. An *Enterobacteriaceae* reduction of log 0.475 was reported from before filtration to after the smallest bag filter. The *E. coli* results showed a 0.43 log reduction after the same bag filter. The HPC test results indicated a 0.4 log reduction after the smallest bag filter. The reductions could be as a result of bacteria being bound or attached to the suspended solids that got filtered out of the water through the bag filter. Following filtration with the 5 µm bag filter, three doses of UV radiation were applied to the water samples using a LP collimated beam UV system. It was determined that the lowest dose applied (20 mJ.cm^{-2}) was sufficient for reducing the *E. coli* and *Enterobacteriaceae* colonies to non-detectable levels. The HPC colonies, were lowered more steadily. This is an indication that there are bacteria in the microbial population that are less sensitive to disinfection with UV radiation. A dose of 60 mJ.cm^{-2} was required to lower the HPC colonies present in the river water to a non-detectable level. There are no guideline limits for

Enterobacteriaceae or HPC, however, in terms of the *E. coli* counts, the water would be deemed microbially safe for agricultural irrigation following treatment with UV radiation.

Overall, the bag filter was successful at improving most of the physico-chemical characteristics. The extremely high TDS content prevented a larger increase in the UVT %. The bag filter was also successful at removing some of the microorganisms present in the river water. The UV results show, again, that this method of disinfection is effective, at laboratory-scale, at producing a consistently safe supply of water. A recommendation for future research would be to repeat this experiment on a river with a different physico-chemical profile. This would provide an indication of the impact of the bag filter on the UVT % of a river with lower TDS content and a higher TSS content. Microbial recovery post-UV radiation was not observed in this study, and due to the consistent presence of *E. coli* and *Enterobacteriaceae* in the river water observed in this chapter as well as the other rivers in Chapter 3, it is recommended that this area of research be analysed in future.

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Chapter 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

General conclusions

Researchers have established that pathogenic microorganisms are commonly the cause of foodborne disease outbreaks (Steele & Odumeru, 2004, Pachepsky *et al.,* 2011, Huisamen, 2012). Pachepsky *et al.* (2011) states that ever-increasing evidence points towards raw fresh produce as the major factor that contributes towards gastrointestinal illnesses. Gerba & Choi (2006) state that irrigation water plays a key role in pathogen transport to consumers of fresh produce via the faecal-oral route. Contamination of surface water occurs more frequently in developing countries which results in decreased water quality and increased risk of disease transmission. In South Africa, surface water is the preferred water source for the irrigation of fresh produce (Singh, 2013). Maree *et al.* (2016) notes that of the available fresh water in South Africa, surface water usage totals 77%. Surface waters in the Western Cape have been the subject of frequent investigation due to the constant presence of faecal contamination, which renders some sources unsuitable for irrigation without pre-treatment (Barnes & Taylor, 2004, Paulse *et al.,* 2009, Lamprecht *et al.,* 2014, Olivier, 2015, Sivhute, 2019). These contaminated surface waters need to be treated to such an extent that it is safe for agricultural irrigation, without the risk of transferring the pathogenic microorganisms to the consumer of the fresh produce.

Locally, and internationally, *Escherichia coli (E. coli)* is frequently used as an indicator of contamination of water, specifically faecal contamination (Britz *et al.,* 2012). This microorganism is included in the guidelines of most countries with limits which need to be complied with in order for the water to be deemed safe for consumption and use. However, foodborne disease outbreaks are not only caused by pathogenic strains of *E. coli,* but also by other pathogens such as *Salmonella* and *Listeria monocytogenes* (*L. monocytogenes)* (Jung *et al.,* 2014). In South Africa, the microbial guidelines for agricultural irrigation are limited to 1 000 colony forming units (cfu) of faecal coliforms *(E. coli)* per 100 mL of water (Department of Water Affairs and Forestry (DWAF), 1996). The potential for bacteria to survive and proliferate in/on the crop becomes a major concern in the case of food safety, particularly in South Africa where a large percentage of the population is immunocompromised as a result of HIV/AIDS (Britz *et al.,* 2012). GreenCape (2020) states that the Western Cape contributes 21% of the country's total agricultural income, and 44% of all exported agricultural products. From a financial perspective, the public consequences of foodborne disease outbreaks linked to contaminated crops, as well as product recalls, can negatively affect the agricultural industry and cause a crippling effect on the economy.

Based on the frequently observed high microbial loads present in Western Cape rivers, Britz *et al.* (2012) has indicated that a 3 – 4 log reduction of faecal coliforms in water intended for agricultural irrigation should be sufficient to meet the guideline limits in water with initial *E. coli* counts in the range of 10^6 cfu.100 mL^{-1}. Physical, chemical and photochemical treatment processes are commonly used to disinfect water, each having their own advantages and disadvantages. The treatment explored in this study, Ultraviolet (UV) radiation, has been investigated by several researchers worldwide (Quek & Hu, 2008, Bolton & Cotton, 2008, Farrell *et al.*, 2018, Gayán *et al.,* 2014). This method of disinfection has proven to be successful across a broad range of microorganisms, with little negative after effects and is therefore, attractive due to the push towards more environmentally friendly treatment methods (Galvéz & Rodrígues, 2010). This is a major advantage over other treatment options, specifically due to the fact that chlorine has become infamous for the production of carcinogenic disinfection by-products (DBPs) in water, as well as the residual chlorine that remains after treatment (Whitby & Scheible, 2004). As chemical treatment methods have been the preferred treatment option for many years (Whitby & Scheible, 2004), environmental microorganisms have developed resistances to this disinfection method, and either require higher concentrations to achieve the same effect or an alternate treatment is required (Momba *et al.,* 2008). Physical treatment methods (slow sand filtration or membrane filtration) can be time consuming and expensive and are limited to the physical size of the pores in the filter, resulting in an often ineffective treatment (Okpara *et al.,* 2011). Physical methods are often used as a pre-treatment step in water disinfection. Most photochemical treatment options, such as Advanced Oxidation Processes or Electron Beam Technology, are expensive and are not user-friendly (Maruthi *et al.,* 2011). Ultraviolet radiation is a simple, yet effective method of disinfection in many industries including water treatment. However, this method of disinfection is not frequently implemented at farm-scale due to chemical treatment methods being the established practice (Olivier, 2015). Studies on the effect of UV radiation used for microbial disinfection commonly involve laboratory-cultured strains of *E. coli* only and low–pressure (LP) UV radiation. This has resulted in a great deal of confusion with regard to dosage requirements for environmental or clinical strains which are often reported as more resistant than laboratory-cultured strains (Hijnen *et al.,* 2006, Chevrefils *et al.,* 2006). No research has been performed in South Africa on the effect of UV radiation on environmental strains of *Salmonella* and *L. monocytogenes* in both water and food. The overall aim of this study was, therefore, to investigate the efficacy of LP UV radiation on water from four different rivers that have varying physical and microbial characteristics, in order to reduce microbial loads for improved crop safety.

The study consisted of three research chapters. The first (Chapter 3) involved the establishment of physico-chemical and microbial profiles of the four selected rivers (Mosselbank, Eerste, Plankenburg and Franschhoek Rivers). The rivers were sampled in the dry, summer season (October 2019 – January 2020). Three doses of LP UV radiation (20, 40 & 60 mJ.cm^{-2}) were applied to the water samples. Microbial tests included Heterotrophic Plate Count (HPC), *Enterobacteriaceae* and *E. coli* counts, as well as presence/absence tests for *Salmonella* species and *L. monocytogenes.* It was established in this chapter that the rivers under investigation frequently exceeded the Irrigation Water Guideline limits for *E. coli* (DWAF, 1996). The food pathogens, *Salmonella* and *L. monocytogenes,* were also present on multiple sampling occasions. Low-pressure UV radiation was effective at reducing the microbial loads to what would be considered safe according to the Irrigation Water Guidelines. No pathogens could be detected after treatment at a dose of 40 mJ.cm^{-2}. The Mosselbank River was deemed the 'worst-case scenario' in terms of microbial loads and physico-chemical characteristics, while the Franschhoek River was classified as the 'best-case scenario'. It was noted that the physical quality of the water did not affect the disinfection capability of the UV radiation, provided that the correct UV dose was applied. In water of low physico-chemical quality, treatment was, however, negatively impacted since extended exposure time was necessary to apply the required dose by LP UV. The turbidity, total suspended solids, total dissolved solids and chemical oxygen demand had the greatest influence on reducing the UV transmission percentage (UVT %) and thereby, increasing exposure time required to apply a specific dose of UV. It was suggested that the use of a pre-treatment step would be advantageous in the case of river waters with high turbidity and suspended solids, which could potentially improve the UVT % and reduce exposure times, ultimately making the process more efficient. Microbial isolates were retained from this study and stored at -80°C for future research.

In the second research chapter (Chapter 4), the isolates from the river waters were analysed in depth in a three-part study. The first involved the lineage-typing of *L. monocytogenes* river water isolates using the PCR-Restriction Fragment Length Polymorphism method set out by Rip & Gouws (2020). It was determined that all *L. monocytogenes* river water isolates belonged to lineage I. This lineage is considered to be the most common cause of listeriosis worldwide (Pirone-Davies *et al.,* 2018). The isolates (*Enterobacteriaceae* and *L. monocytogenes*) were then subjected to antimicrobial susceptibility testing. *Enterobacteriaceae* were also tested for the presence of extended-spectrum beta-lactamase (ESBL) producers. A total of 90% (n=9) of the river water isolates tested were multidrug resistant. Ampicillin resistance was reported for 100% of the *Enterobacteriaceae* isolates (n=5) and 80% (n=4) of the *L. monocytogenes* isolates. None of

the *Enterobacteriaceae* isolates were ESBL-producers. In the third study, the isolates were inoculated into sterile river water at concentrations higher than would naturally be found in the environment (approximately 10^8 cfu.mL^{-1}) and exposed to the same three doses of UV radiation as before (20, 40 & 60 mJ.cm^{-2}). It was established that LP UV radiation was not effective at reducing the microbial levels to those that would be considered as safe, when the initial microbial loads are higher than 10^8 cfu.mL^{-1}. The most UV-resistant strain was an *E. coli* isolate from the Franschhoek River. This river was described in the previous study (Chapter 3) as the 'best-case scenario'. The environmental *L. monocytogenes* and *Salmonella* river isolates tested in this study required at least 40 mJ.cm^{-2} of UV radiation to reduce the microbial loads to non-detectable levels. Therefore, these pathogenic microorganisms (*Salmonella* and *L. monocytogenes*) require lower doses of UV radiation than *E. coli* (required > 60 mJ.cm^{-2}) at the investigated microbial loads. This is in contrast to literature that states that, in general, pathogenic microorganisms are more resistant to disinfection than non-pathogenic ones (Gayán *et al.,* 2014). From this chapter's results, it was concluded that the river water isolates carry resistance genes to multiple antimicrobials as well as show resistance to disinfection with UV radiation at high microbial loads. This was noted specifically with regard to the *E. coli* isolates (FR-01 and MR-01) that required at least 60 mJ.cm^{-2} of UV radiation for disinfection. It is evident that these microorganisms pose a potential risk for human and animal health if consumed.

In the third research chapter (Chapter 5), a bag filter was tested to determine the effect of this pre-treatment step on the physico-chemical profile of the river water prior to UV radiation. Since the results from the first research chapter (Chapter 3) indicated that the river with the worst physico-chemical profile was the Mosselbank River, water was sampled from this river and run through bag filters of varying pore sizes (5, 20, 50 & 100 µm). Microbial and physico-chemical characteristics were analysed before and after each pore size. It was noted that the bag filter with the pore size of 5 µm yielded the best results, without compromising the flow rate. The total dissolved solids (TDS) content (avg. 715.33 mg.L^{-1}) even after the bag filter with the smallest pore size was used, was not considered acceptable for agricultural irrigation purposes according to the guideline limit of 260 mg.L^{-1} (DWAF, 1996). Heterotrophic Plate Count, *Enterobacteriaceae* and *E. coli* microbial loads were reduced by 0.4, 0.475 and 0.43 log cfu.mL^{-1}, respectively, from before treatment to after the 5 µm bag filter was used. A large improvement (32.4%) was noted for the total suspended solids (TSS) content of the water, following the use of the bag filter. A 1.83% improvement was reported for the UVT % of the water sample after the 5 µm bag filter was used. It can be speculated that this increase could have been a much larger improvement in the UVT % if the TDS content of the river water was lower than the extreme levels recorded for the Mosselbank River. This bag filter (5 µm) was

selected as the most effective pore size for future experiments. River water samples that were filtered through the 5 µm bag filter, were also exposed to the same three LP UV doses as before. The lowest dose (20 $mJ.cm^{-2}$) was sufficient for reducing the *E. coli, Enterobacteriaceae, Salmonella* and *L. monocytogenes* loads to non-detectable levels. The HPC loads lowered more steadily, and required at least 60 $mJ.cm^{-2}$ to reach non-detectable levels. This is an indication of a microbial population that is less sensitive to UV disinfection, and agrees with previous findings in the current study and with literature (Bester, 2015, Olivier, 2015).

Recommendations for future research

Results from the first research chapter (Chapter 3) indicated that LP UV radiation is effective at reducing the microbial loads to acceptable levels, as well as reducing pathogenic microorganisms to non-detectable levels. It was established that the physical quality of the river water influenced the length of exposure times required to achieve a specific dose, but did not have a negative impact on the final level of disinfection. Essentially, it was possible to achieve a standard of water that would be considered microbiologically safe for use, according to the Irrigation Water Guidelines (DWAF, 1996), no matter what the initial physical quality of the water was. It was suggested that a pre-treatment step could be beneficial for treatment efficiency by reducing suspended solids and improving UVT %.

In the second research chapter (Chapter 4), it was established that multidrug resistance was reported for 90% of microbial isolates (n=9). In 2014, the World Health Organisation (WHO) developed a global action plan in response to the rapid increase in antimicrobial resistant bacteria, in which one of the strategies included continuous research and surveillance of antimicrobial resistance in environmental isolates in order to curb the spread of these pathogenic microorganisms. It is recommended that more river water isolates (*Enterobacteriaceae* and *L. monocytogenes*) be tested, to understand how these organisms disseminate and spread resistance in river waters, and determine the possible effect of seasonal changes on resistance patterns. None of the *Enterobacteriaceae* river water isolates were ESBL-producers, however, it is recommended that more isolates are obtained from these rivers and that testing is performed for the presence of both ESBL-producers as well as AmpC-producing genes. This would fill the knowledge gap in this area, as there is currently no research that has been performed on the presence of AmpC-producers in irrigation water sources in South Africa. Thomson (2010) states that multidrug resistances are commonly associated with plasmid mediated AmpC β-lactamases in *Enterobacteriaceae.* AmpC-producers are not inhibited by β-lactam inhibitors and are able to confer resistance to Cephamycins (Rodríguez-Baño *et al.,* 2018). Tepeli and Zorba (2018) state that AmpC β-lactamases are resistant to a broader range of antimicrobials than ESBL-producers. For this

reason, it is recommended that research be performed in this area. Another recommendation for future research based on the results of the third study of the second chapter (Chapter 4) was to determine the resistance profiles of isolates to UV radiation after previously being exposed to UV radiation.

In the third research chapter (Chapter 5), it is recommended that river water with a different physico-chemical profile, specifically one with a lower TDS content, should be investigated using the same bag filter pore sizes. The results indicated that filtration improved TSS levels more than TDS, and it might be that in water with higher a TSS and lower TDS content, a greater improvement in UVT % might be observed. Investigating the same pore-sized bag filters on a range of different river water profiles, therefore, enables for a more accurate determination of the limitations for practical application of this pre-treatment technology. This could enable the intended user of the UV radiation to apply the most effective treatment, which takes a wider variety of profiles into consideration.

Based on the results obtained in the first and second research chapters (Chapters 3 and 4), it is recommended that HPC isolates are obtained from the river water, after UV radiation, and identified. This would determine which strains are contributing to the UV resistance observed in the river waters that may present a food safety risk to fresh produce consumers. Screening for Shiga-toxin producing *E. coli* (STEC) testing is also recommended. An alarmingly low infectious dose has been reported for STEC, ranging between 10 – 100 cells required to cause infection (Etcheverría & Padola, 2013, Karmali, 2004). If *E. coli* cells are able to survive UV radiation, it is important to determine if the surviving strains are STEC or commensal *E. coli* so as to determine the potential risk associated with the water that carries low *E. coli* loads after treatment.

In this study, the effect of microbial recovery, including photoreactivation and dark repair, was not investigated. Zimmer & Slawson (2002) state that microbial recovery following radiation with LP lamps is a cause for concern. Photoreactivation (light-dependent reactivation) is particularly problematic in areas where sunlight is plentiful and temperatures range between 23 - 37°C (Quek & Hu, 2008). This is, therefore, recommended for future research in order to ensure that the microorganisms inactivated during treatment have not recovered to such an extent that it might still present a health risk to the consumer following UV treatment.

The *E. coli* loads present in the examined rivers called for log reductions exceeding 3-log in order to produce water that is fit for agricultural irrigation, based on guideline limits. If treated effectively and completely with UV radiation, the risk of microbial contamination from irrigation water is low. Due to the fact that pathogenic microorganisms, such as *L.*

monocytogenes and *Salmonella* spp. were frequently identified in the various rivers investigated, these pathogens may present consistent food safety risks for the consumers of fresh produce which was irrigated with this water. It is recommended that the relevant authorities are informed of the potential risk associated with these pathogens. Antimicrobial resistant microorganisms that have been identified in the rivers investigated may indirectly place great pressure on the clinical industry as treatment options can become limited for infections caused by multidrug resistant strains. It is imperative that the occurrence of these microorganisms be routinely determined in order to monitor their environmental dissemination.

In conclusion, at the investigated doses, LP UV radiation is able to seamlessly provide a consistent level of disinfection and shows promise as a method for irrigation water disinfection. Based on the results obtained in this study, the transition from LP UV treatment at laboratory-scale to the medium-pressure UV treatment pilot-plant can be made to determine the feasibility of upscaling.

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